DEVELOPMENT OF SAMPLING STRATEGIES FOR MANAGEMENT STUDIES OF *Hoplolaimus galeatus* ON ST. AUGUSTINEGRASS (*Stenotaphrum secundatum*)

By

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DEVELOPMENT OF SAMPLING STRATEGIES FOR MANAGEMENT STUDIES OF *Hoplolaimus galeatus* ON ST. AUGUSTINEGRASS (*Stenotaphrum secundatum*)

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The relationship of the lance nematode *Hoplolaimus galeatus* (Cobb) Thorne to St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] was explored to develop sampling strategies and operational parameters which could be used in management studies. The research consisted of five parts: host suitabilities of commonly available St. Augustinegrass varieties to *H. galeatus*, extraction losses and core size recovery from two differently sized sampling units, determination of an optimal sampling plan, monthly population fluctuations in relation to sampling and weather, and testing of the sampling plan.

Seven commonly available varieties of St. Augustinegrass had similar host suitabilities to *H. galeatus*. Clipping weights of inoculated plants decreased during the trials and differed among groups of St. Augustinegrass but not among ploidy levels.

Extraction losses by a modified sieving-centrifugal flotation method were greater for a 10.2-cm-d sampling unit than for a 2.2-cm-d unit. The large unit lost a greater proportion of nematodes than did the small unit. Losses were dependent on nematode type, life stage, and processing step.

The large unit recovered a greater frequency of nematodes than did the small, so fewer large units were required to estimate the population with a given level of confidence. However, processing losses and times expended were so great for the large unit that it was discarded.

Two differently managed St. Augustinegrass areas, one with a high density, the other with a low density, were sampled monthly. Population specific growth rates (maximum of 4.3) of H. galeatus seemed related to optimum growth periods of St. Augustinegrass. The age structure showed the adults to be longer-lived and that the best time to try to influence population growth would be January to April. Highest densities were in the 0-7.6 cm soil profile during the spring and summer, but changed to the 7.7-23.0 cm depths during the winter. Smallest sample sizes for population estimates would have been required in July through December.

Stratification on the basis of surface criteria proved efficient. All life stages of H. galeatus had similar β values of Taylor's power law.

CHAPTER I
INTRODUCTION

Urbanization is rapidly altering Florida's environment. Associated with urbanization are plantings of lawns and turf, a system found by Falk (1976) to be energy intensive. The maintenance costs for Florida turf during 1974 were estimated to be equivalent to 28% of all energy consumed in Florida's agricultural production (Busey and Burt, 1979). Due to Florida's rather warm mean temperature, and the droughty sandy soils which exacerbate otherwise minor problems, pest control may comprise a larger proportion of these maintenance costs than in other areas of the country. Among nonathletic turf plantings in Florida, St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is the most popular, probably because of its wide range of utility (e.g. traffic tolerance, coverage, color and cutting characteristics) in relation to its maintenance costs (Busey and Burt, 1979). Of all turf samples submitted to the University of Florida Nematode Assay Lab for analysis of a suspected nematode problem during 1985, 60% were from St. Augustinegrass lawns and 72% of these had levels of lance nematodes, *Hoplolaimus* spp., thought to be great enough to have been contributing to their unthrifty nature.

The urban lawn is a highly productive perennial ecosystem (Falk, 1976) which is managed for aesthetic values rather than for harvestable yields. Consequently, economic considerations are often secondary and inputs in time and energy can be high (Falk, 1976). The permanence and aesthetic nature of lawns prevent the use of many pest management practices commonly utilized for nematode management in field crops.

Chemical control may also not be a viable alternative in some situations. The liability of applying nematicides in high population density areas may be rather great. Additionally, the few chemicals currently registered for use on established turf are often ineffective against lance nematodes (Perry et al., 1970; Perry and Winchester, 1969), and may be environmentally risky because of Florida's high water table and sandy soils.

Environmental and practical concerns justify exploration of different approaches to managing nematode populations. Appropriate manipulations of the many inputs necessary for even minimal turf maintenance may provide the management tools.

The objective of this study was to develop sampling tools and some preliminary data which would permit detailed exploration of _H. galeatus_ populations associated with St. Augustinegrass for the investigation of sound management practices. Since _Hoplolaimus galeatus_ is a soil borne plant parasite and is distributed in time as well as in space, problems of extraction from soil and temporal distribution as related to sampling, intertwine in the project.

CHAPTER II
LITERATURE REVIEW

Knowledge of a pest's population density is basic to the application of integrated pest management (Metcalf and Luckmann, 1975). It is from these data that economic thresholds are developed and from which decisions can be made as to appropriate actions.

It is impractical to count all the pests in a particular area of interest, so most often their density is estimated from a number of counts made from different locations. This process is called sampling. Since only a few of all possible locations within the area are sampled, it is important that the sample locations be representative of the pest or inaccurate population estimates will result. Imprecise estimation of the pest's density may result in loss to the grower, either by not taking a management action when one was justified or by taking an action that was not necessary, thereby wasting resources and potentially damaging the environment. An overly precise estimation of the pest's density results in wasted effort and resources. Proper choice of sample locations will permit the fewest possible samples to be drawn while providing the required degree of reliability.

Selection of sample locations should be guided by knowledge of the pest's biology, life history, and ecology (Southwood, 1978). Since pest numbers vary with time as well as in the three spatial dimensions, these components should also be considered when sampling. Major factors governing plant parasitic nematodes are the growth cycles and health of their host, especially its roots (Wallace, 1978).

The Host

The genus Stenotaphrum (Gramineae: Paniceae) is primarily tropical but several of its members are widely distributed (Sauer, 1972). One of these is Stenotaphrum secundatum (Walt.) Kuntze, St. Augustinegrass, which is often planted as a lawn or pasture grass in the warmer regions of the world (Sauer, 1972). A classification of common varieties of this species by Busey et al. (1982) recognized five different groups.

St. Augustinegrass is a popular Florida lawn grass because of its warm season adaptation and its range of utility (e.g. coverage, traffic tolerance, and color) over a wide possible range of management inputs (Busey and Burt, 1979). When planted in replicated field plots, Busey and Augustin (1980) judged that the Floratam variety was the best choice for coverage, color, and chinch bug resistance. Other common varieties include Roselawn, Bitterblue, Floratine, Seville, and a new release, Floralawn (FA108).

Optimum root growth of St. Augustinegrass occurs from 29.4-35 C, but may continue into late fall with 10 cm soil temperatures as low as 10 C, well after shoot growth has ceased (Beard, 1982). At colder temperatures the grass enters a dormant period. During winter dormancy the roots apparently remain functioning since they appear white and healthy, but no growth occurs (Beard, 1982). Upon the arrival of spring, the grass begins to green and the roots of this perennial grass exhibit annual behavior--they die and are totally replaced (Beard, 1982).

When spring soil temperatures (10 cm depth) reach ca. 17 C area, the turf starts to green up and about four days later, the root system browns and dies, often within 24 hours (Beard, 1982). This results in a ca. two week period when the root system is not functionally capable of supporting normal growth. During this period, susceptibility to damage by factors causing plant stress may increase (Beard, 1982).

Major factors influencing the culture of turfgrass in Florida include water use and irrigation. Water stress, basic rooting patterns and photosynthetic adjustment in St. Augustinegrass to water stress have been studied by Peacock (1981). A model of the rate of vegetative propagation of several turfgrasses has been published by Busey and Myers (1979).

The Pest

The genus Hoplolaimus Daday, 1905 is based upon a single specimen recovered from a mudhole on an island in the Paraguay River, South America. The specimen was poorly preserved and described, which led to later taxonomic confusion. Cobb (1923) finally redescribed the genus and started taxonomic house cleaning. Andrassy (1958) redescribed the type species of the genus (H. tylenchiformis Daday) from the original specimen and synonymized the ubiquitous North American species H. coronatus Cobb with it.

In revising the genus, Sher (1961) disagreed with this lumping and split out H. coronatus once again, but recognized its synonymy with Nemonchus galeatus (Cobb, 1913) and renamed it Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935.

This nematode is 1.24-1.94 mm in length and has an 'a' value of 25-34. A key diagnostic character is the lip region, which possesses 4-5 lip annules. The cephalic framework and stylet are strong, and the stylet knobs project anteriorly in a tulip shape (Sher, 1963). Various aspects of the detailed morphology of H. galeatus have been studied by Hirschmann (1959), Coomans (1962) and Hogger and Bird (1974).

The life history of H. galeatus has not been studied in detail, but those of several related species have been. Female Hoplolaimus indicus Sher lay about three to four eggs per day, but the total number laid in a lifetime is unknown (Dasgupta et al., 1970). This is similar for Hoplolaimus *seinhorsti* Luc (Rajaram, 1983). The time from egg deposition to hatch for *H. indicus* at 28 to 32 C is about 72 to 96 hours, and the total time from egg to egg is 27 to 36 days (Dasgupta et al., 1970). The life stages are separable by total length. For *Hoplolaimus columbus* Sher, the time from egg-laying to hatch is twelve days (Fassuliotis, 1975). Rivera-Camarena (1963) studied the hatching of *H. galeatus* at three temperatures in water drops. He determined that the time to hatching was temperature dependent.

Environmental factors that influence the life history of *H. galeatus* include the host, the soil, and the soil pH. The optimal temperature for reproduction of *H. galeatus* on dent corn was 27 C. The upper temperature at which reproduction occurred on this host was 30 C, but it was 32 C on clover. On both hosts, the lower limit was 20 C (Willut and Malek, 1981).

Ahmad and Chen (1980) found a significant three-way interaction among soil type, host (bean and barley) and pH influencing reproduction of *H. galeatus*. Sandy loam or silty loam supported greater numbers than did loamy sand. This agrees with data on *H. indicus* which shows greater reproduction in sandy loam than in sand or clay soils across a range of temperatures (Gupta and Atwal, 1971). In Illinois farm soils, however, *H. galeatus* distributions do not seem to be influenced by soil type (Schiel, 1971) but do seem to be related to exchangeable bases and cation exchange capacity.

Organic amendments may influence Hoplolaimus spp. Hutchinson et al. (1960) noted low populations of H. tylenchiformis in areas of the field where pumpkins had been allowed to rot the year before. Activated sewage sludge also seems to reduce H. galeatus populations (Nutter, 1958).

H. galeatus on soybean reproduce maximally at pH 6.0 (Burns, 1971). Maximal egg deposition for H. indicus, however, was at pH 7.0. No reproduction of this nematode occurred at pH 2.5 or 10.5, but some feeding did occur (Dasgupta et al., 1970).

Populations of H. galeatus fluctuate seasonally (Chapman, 1976; Lucas et al., 1978). Both Lucas et al. (1978) and Chapman (1976) studied fluctuations of H. galeatus associated with different cool season turf grasses. These authors found that populations were lowest during August and were highest earlier in the year. Lucas et al. (1978) related these changes to the growth periods of the host and said June to July or October to November would be the best time to sample. Chapman (1976) observed that the composition of the population, 46% juvenile, 18% males and 36% females, remained relatively constant throughout the year. These population dynamics may also be influenced by rainfall since H. tylenchiformis populations seem to increase after periods of rainfall (Minton et al., 1960).

The Problem

Hoplolaimus galeatus was first associated with unthrifty St. Augustinegrass in 1953 by Kelsheimer and Overman (1953), who also outlined many of the most important sanitation practices still recommended today for severe situations calling for replanting. Surveys since then (Christie et al., 1954; Good et al., 1956; Parris, 1957; Perry et al., 1970) have confirmed the extent of the association.

On cotton, alfalfa and bermudagrass, lance nematodes feed ectoparasitically or they may feed and burrow inside the root cortex and feed on the stele (Krusburg and Sasser, 1956; Ng and Chen, 1985; Rivera-Camarena, 1963). They have been classified as moderately pathogenic to turf (Perry et al., 1970).

The frequency of the St. Augustinegrass-lance nematode association, the generally sandy soils of the southeast coastal plain, the frequent failure of chemicals to reduce their numbers while reducing competing phytoparasites (Perry et al., 1970; Perry and Winchester, 1969) and the sensitivity of Florida's groundwater to pesticide contamination while the public demands green lawns, combine to create a need for better understanding of this system. Exploration of the population characteristics exhibited by *H. galeatus* and the factors which govern them are cornerstones which must be laid before widely applicable management practices can be implemented.

The Study Tools

Accurate and reliable estimation of nematodes associated with turf are necessary for determination of economic thresholds and for management decisions based on nematode assay results (Barker and Nusbaum, 1971). Any sampling plan should use knowledge of the target's life history, biology and ecology to make it more effective (Southwood, 1978). This knowledge can be incorporated into all stages of the sampling process--accounting for the distribution of the pest, developing a sampling plan, and optimizing the cost of the program. If the process of sampling is too expensive or inaccurate, then the sampling plan should be redesigned (Ruesink and Kogan, 1975).

Until 1971, few sampling studies for nematodes had been performed (Barker and Nusbaum, 1971), other than for cyst nematodes (Anscombe, 1950; Church et al., 1959; Fidler et al., 1959; Widdowson, 1962).

Distribution

Nematodes are distributed horizontally, vertically and temporally in the soil. The nature of these distributions are influenced by host, soil, and other environmental characteristics (Barker and Nusbaum, 1971; Wallace, 1978). Many workers, starting with Cobb (1918), have noted the aggregated distribution of soil nematodes. This distribution is often mathematically described by a negative binomial distribution (Anscombe, 1950; Barker and Nusbaum, 1971; Goodell and Ferris, 1980; McSorley and Parrado, 1982a).

Description of the distribution of an organism is important in designing sampling schemes. This information aids in the development of data transformation to meet the assumptions of parametric statistical tests, in the development of sampling techniques, and in comparing interspecific distribution differences. The mathematical description of a population's distribution may change with its density, with time (Taylor, 1984), or with the size of the area sampled, as shown for H. galeatus in Indiana (Alby et al., 1983). In addition, distribution of some nematodes may not fit a standard mathematical description (McSorley et al., 1985).

Because of these problems, Taylor's power law (Taylor, 1961) has come under increased scrutiny for development of sampling plans (Finch et al., 1975; Ferris, 1984a). It relates the mean ($\overline{x}$) of a group of samples to the variance (s^2). The relationship is expressed as $s^2 = ax^b$ or $\log s^2 = \log a + b \log \overline{x}$, where 'a' and 'b' are constants. The constant 'b' is an index of dispersion, which is characteristic of the species (Taylor, 1961). The constant 'a' is both a sampling factor and is related to the environment (Taylor et al., 1983). Taylor's power law is otherwise useful in determining a transformation for parametric analyses (Taylor, 1961).

Sampling

Sampling decisions consist of the selection of the sampling universe, the sampling unit, number of samples, sampling pattern and the economics of sampling (Southwood, 1978). If multiple cores are to be bulked, (a common practice in sampling for soil pests since a better estimate of the population for lower costs is obtained but with loss of information about the population's variation) (Southwood, 1978), decisions influencing this must also be included (Francl, 1986).

The selection of a sampling unit is an important aspect in the design of a sampling plan (Andrassy, 1962; Southwood, 1978). At least two sizes of sampling units should be used for comparative purposes. One of these should be near the smallest size for meaningful sampling of the habitat since, for the same costs, greater reproducibility may be gained by taking many small samples than by taking fewer larger samples. On the other hand, larger sampling units yield counts with fewer zero observations and may be of greatest value when pest densities are low (Southwood, 1978).

If a sampling program is to be transferable to other situations, guidelines as to the sampling pattern and the number of samples per unit area are necessary. For example, removal of 20-100 cores in a zig-zag pattern from areas up to five acres was recommended by Barker and Nusbaum (1971). Removal patterns based on edaphic or cultural variables were also mentioned as being satisfactory. Partitioning a variable area into more homogeneous subunits is called stratifying (Cochran, 1977). Goodell and Ferris (1981) showed that this practice is desirable in at least some situations where soil types are known.

Determination of sample size is necessary to ensure proper estimation of the target population. McSorley and Parrado (1982b) determined the optimal sample size for a number of different nematode species associated with trees in fruit groves. Goodell and Ferris (1981) investigated a number of sampling patterns and sample sizes for nematodes associated with alfalfa. More such examples exist for cyst nematodes (e.g. Anscombe, 1950; Church et al., 1959; Widdowson, 1962).

The economics of sampling and extraction are important determinants in optimizing the sampling plan (Cochran, 1977; Goodell and Ferris, 1981; McSorley and Parrado, 1982b). A good sampling plan is a compromise between the time (costs) and the precision required. In order to be an optimal sampling plan, the cost of it must be minimized relative to the level of desired precision (Yates and Finney, 1942).

Extraction from the soil

The investigation of plant parasitic nematodes has always been hampered by lack of a simple and efficient technique for removing these organisms from the soil

(Christie and Perry, 1951). Not much has changed since Christie and Perry penned these words over three decades ago.

Recent work on extraction losses associated with various techniques and their component steps show efficiencies ranging from about 3% to 45% (Viglierchio and Schmitt, 1983a, b; Viglierchio and Yamashita, 1983). Nematode recovery seems to depend upon the particular nematode species, the soil type and condition, and the amount, type and concentration of the extraction solutions used (Ayala et al., 1963; Barker et al., 1969a, b; Rodriguez-Kabana and King, 1975; Viglierchio and Schmitt, 1983a, b; Viglierchio and Yamashita, 1983). These extraction dependent losses have prompted the suggestion that extraction calibrations be conducted before undertaking the sampling (Goodell, 1982).

CHAPTER III
REPRODUCTION OF Hoplolaimus galeatus ON SEVEN ST. AUGUSTINEGRASS (Stenotaphrum secundatum) VARIETIES

Association between lance nematodes (Hoplolaimus spp.) and unthrifty St. Augustinegrass (Stenotaphrum secundatum (Walt.) Kuntze) lawns was first made in 1953 (Kelsheimer and Overman, 1953). Subsequent surveys (Good et al., 1956; Perry and Good, 1968; Perry et al., 1970; Perry and Winchester, 1969; Whitton, 1956) have frequently associated Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935 with problem lawns in Florida.

Lance nematodes often feed endoparasitically on St. Augustinegrass (Good et al., 1956; Perry and Good, 1968; Whitton, 1956). On cotton, alfalfa and bermudagrass (Krusberg and Sasser, 1956; Ng and Chen, 1985; Rivera-Camarena, 1963), they tunnel and feed in the cortex and feed on the vascular cylinder. In their 1956 study of Hoplolaimus galeatus on cotton, Krusberg and Sasser (1956) concluded that host symptoms were accentuated by dry conditions and that greenhouse-grown plants supplied with adequate moisture and nutrients can tolerate rather high populations. These observations probably apply to the relationship of this nematode to St. Augustinegrass, on which it has been classified as moderately pathogenic (Perry et al., 1970).

The lance nematode poses a frequent and seemingly unmanageable problem in many St. Augustinegrass lawns, because of its widespread distribution, the generally sandy soils of the southeast coastal plain and the frequent failure of chemicals to reduce their numbers while controlling competing phytoparasites (Perry et al., 1970; Perry and Winchester, 1969). Sensitivity of Florida's groundwater to contamination by pesticides raises the question of continued availability of nematicides for this purpose.

The discovery of St. Augustinegrass varieties resistant to Southern chinch bug (Reinert and Dudeck, 1974) extends a ray of hope that some resistance to the lance nematode might be found. The objective of this study was to survey the ability of commercially available St. Augustinegrass varieties to support an increase in the population of lance nematodes.

Materials and Methods

Three different trials were conducted. In each trial, eight nodes of each of seven St. Augustinegrass varieties were planted separately in uniform 15.2-cm-d clay pots containing steam pasteurized Arredondo fine sand (ca. 93% sand, 5% silt, 2% clay, 0.1% organic matter, pH 7.5). Turf nodes were added or subtracted from the pots to keep their density constant while covering the soil surface. The pots were fertilized once a week with 200 ml of a 1% 20-20-20 (N-P-K) solution.

Of the three different trials, two were kept in the greenhouse and the third was transferred from the greenhouse one week after the pots had been infested, to 20-cm-d X 76cm deep polyvinyl chloride outdoor microplots containing methyl bromide treated Arredondo fine sand. The three trials were staggered over 10 months (February-May, July-October, August-November). The design for all trials was a randomized complete block with seven commonly available varieties of St. Augustinegrass (Table 3-1) and five replications, except in the second greenhouse trial in which there were four replications. The experimental unit was a 15.2-cm-d clay pot, which in the case of the third trial became a microplot.

The pots were infested by pipetting equal numbers of Hoplolaimus galeatus, grown on sorghum (Sorghum bicolor [L.] Moench, Dekalb hybrid forage sorghum), into five 2.5 cm deep holes to obtain a density of 30 lance nematodes per 100 cm^3 of soil. This infestation level was chosen on the basis of previous greenhouse work which indicated that a rapid population increase could be expected at this P_i.

Plants were clipped to a uniform height prior to commencing the trials and cut similarly as needed during each trial. The clippings were placed in labeled paper bags and dried at 60 C for two days before cooling in a desiccator and weighing.

Harvest at 3.5 month after infestation included final clipping weights, visual ratings, four lance nematode lifestages (2nd stage juveniles, 3+4 stage juveniles, males and females) per 100 cm^3 soil as extracted by modified sieving-centrifugation (Ayoub, 1980), and the number of lance nematodes per gram of dry root. Nematodes were extracted from the roots using the pie pan method with Scottiesᴿ tissue (Viglierchio and Schmitt, 1983a) for 24 hours at room temperature. After extraction, dry root weights were determined by drying at 60 C, cooling in a desiccator and weighing. Appropriate data analyses were performed using SAS (SAS Institute Inc., 1982a), and mean separation by the Waller-Duncan test at the 5% alpha level.

Results

Combined analysis of variance (Tables 3-2, 3-3) indicated that the three trials varied significantly ($P \leq 0.0001$). The absence of variety X trial interactions (Table 3-2, 3-3) which would have indicated seasonal effects and have necessitated separate analyses for the three trials, allowed the summary analyses presented here, except where specifically noted.

There were no differences among varieties in the numbers of any lance nematode lifestage or in the total population (Table 3-3). The P_f/P_i ratios averaged over the three trials are presented in Table 3-4. Differences in the P_f/P_i ratios for soil-inhabiting, root dwelling or total lance populations were not found.

There were three clipping times in each trial. Slight and variable differences were seen in the clipping weights of the first and second cuttings of the three trials but these stabilized into distinct and constant differences by the third cutting (Table 3-5). Total clipping weight summed over the three times also differed ($P \leq 0.05$) (Table 3-5).

While significant correlations were observed between the number of soil-dwelling adults and root-inhabiting 3+4 stage juveniles and adults, the level of correlation was low (Table 3-6). Juveniles were three times (17/g dry root) as prevalent inside the root as the next most frequent lifestage, the females (5/g dry root).

Linear contrasts comparing diploid (Roselawn, Seville) versus triploid (Bitterblue, Floralawn, Floratam, Floratine) varieties showed no difference in final clipping weights whereas the Bitterblue (Bitterblue, Floratine) and the Roselawn-Floratam groups (Busey et al., 1982) (Roselawn, Floratam, and Floralawn) differed significantly at the final clipping time ($P \leq 0.005$) (Table 3-7).

Minor contamination with Paratrichodorus spp. occurred in the outdoor microplot test. Differences in population densities among the seven varieties were found and separation of the means is given in Table 3-8.

Discussion

Lance nematodes increased their numbers equally on all seven of the St. Augustinegrass varieties tested (Table 3-4), indicating similar host suitabilities.

The amount of leaf tissue produced by these varieties changed within each trial (Table 3-3). At the final cutting time, the Roselawn-Floratam group produced more than did the Bitterblue group (Table 3-7). These differences may reflect better adaptations of these varieties to conditions of the trials, or to some other factor (e.g. lance nematodes). These speculations can not be addressed by this study. It is informative to note, however, that there is a negative correlation between first year yields of the pasture grasses, Lolium spp. and their persistence (Snaydon, 1984).

Dr. Phillip Busey (personal communication) has observed that after three years, plantings of diploid varieties seem to melt out relative to triploid varieties. Genetic variation in grasses (e.g. polyploidy; Jackson, 1976) has been related to success under herbivory (Antonovics, 1984; Jefferies, 1984). The varying gene expressions made possible by polyploidy (Jackson, 1976) may produce a mosaic of minority genotypes which could escape pathogen pressure (Antonovics, 1984). The diploid vs. triploid contrast of this study did not support the hypothesis of multiple-genome superiority under lance nematode pressure.

The contamination of fumigated soil with low numbers of Paratrichodorus sp. is not uncommon in Florida (Perry, 1953). Significant differences in their numbers on the seven varieties of the microplot trial should provide stimulus for further investigation.

Table 3-1. St. Augustinegrass varieties tested, their chromosome numbers and group to which they belong.

Variety	Chromosome number[Y,Z]	Group[Z]
Bitterblue	30	Bitterblue
Common	unknown	unknown
Floralawn	32	Roselawn-Floratam
Floratam	32	Roselawn-Floratam
Floratine	30	Bitterblue
Roselawn	18	Roselawn-Floratam
Seville	18	Dwarf

[Y] See Busey, 1980.

[Z] See Busey et al., 1982.

Table 3-2. Summary analysis of variance table for all three trials of seven St. Augustinegrass varieties for dried clipping weights at three cutting times.

		Clipping time - weight in grams							
		1		2		3		Final	
Source	D.F.[z]	F	P>F	F	Pr>F	F	Pr>F	F	Pr>F
Trial	2	14.8	0.001	154.9	0.0001	168.4	0.0001	6.0	0.01
Rep (Trials)	11	5.1	0.0001	0.7	0.7	1.8	0.19	3.8	0.0005
Varieties	6	2.1	0.07	1.7	0.1	8.9	0.006	2.9	0.02
Trial (Varieties)	12	0.9	0.6	1.3	0.2	3.8	0.06	0.8	0.7
Error (MSE)	65	7.2		2.5		0.1		9.9	
Total	96								

[z] Five replications in trials 1 and 3, four replications in trial 2.

Table 3-3. Summary analysis of variance table for all three trials of seven St. Augustinegrass varieties for final lance nematode numbers.

		Lance nematode life stages (soil)									
		J2		J34		Male		Female		Total	
Source	D.F.[z]	F	P>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Trial	2	5.86	0.005	10.35	0.0002	55.43	0.0001	37.3	0.0001	26.71	0.0001
Rep (Trial)	11	0.90	0.54	3.94	0.0006	1.19	0.3	0.87	0.6	1.92	0.06
Varieties	6	0.54	0.75	1.23	0.3	1.87	0.1	1.61	0.2	1.63	0.2
Trial (Varieties)	12	0.49	0.88	0.7	0.7	0.96	0.5	1.61	0.1	0.87	0.6
Error (MSE)	65	415.12		1262.0		399.4		1331.0		8236.1	
Total	96										

[z] Five replications in trials 1 and 3, four replications in trial 2.

Table 3-4. P_f/P_i values for soil-inhabiting, root-dwelling and all *H. galeatus* on seven varieties of St. Augustinegrass over all three trials.

Variety	Soil	Root	Both
Bitterblue	7.3	1.0	8.3
Common	6.2	1.2	7.3
Floralawn	7.3	1.3	8.5
Floratam	6.0	1.0	7.0
Floratine	7.7	0.9	8.6
Roselawn	5.1	0.6	5.7
Seville	6.1	0.8	6.9

Table 3-5. Dried clipping weights (g) summed over three cutting times and for each time, for seven St. Augustinegrass varieties infested with lance nematodes. Data combined over three trials.

	Summed Weights	Time 1	Time 2	Time 3
	means (g)^z	means (g)	means (g)	means (g)
Roselawn	12.3 A	8.7 A	2.6 AB	1.0 B
Floratam	11.3 AB	6.6 AB	3.4 A	1.3 A
Seville	9.8 ABC	6.2 B	2.6 AB	0.9 B
Floratine	8.6 BC	5.6 B	2.6 AB	0.6 C
Floralawn	8.6 BC	5.6 B	2.1 AB	1.0 B
Bitterblue	8.3 C	5.2 B	2.1 AB	0.9 B
Common	8.2 C	5.9 B	1.7 B	0.6 C

[z] Means of varieties (14 observations over the trials) in columns followed by the same letter do not differ according to the Waller-Duncan test.

Table 3-6. Correlations (r/p>F) between soil-dwelling and root-inhabiting life stages of *Hoplolaimus galeatus*.

Soil	Root				
	J2	J34	Males	Females	Total
J2	-.06	.04	-.03	.16	.064
	.59	.69	.80	.14	.54
J34	-.15	-.004	-.02	.13	.02
	.16	.97	.83	.20	.84
Males	-.002	-.40	-.22	-.09	-.34
	.98	.0001	.037	.39	.001
Females	-.04	-.36	-.20	-.10	-.31
	.70	.0005	.06	.36	.0026
Total	-.08	-.25	-.15	-.01	-.20
	.43	.02	.15	.91	.06

Table 3-7. Final clipping weights (g) produced by the members of the Roselawn-Floratam group and the Bitterblue group and the linear contrast statistics showing the differences between the two groups.

Roselawn-Floratam Group		Bitterblue Group	
Roselawn	12.3	Bitterblue	8.3
Floratam	11.3	Floratine	8.9
Floralawn	8.6		
Contrast:	Sum of Squares 2.10	F=13.08	Pr>F 0.0005

Table 3-8. *Paratrichodorus* sp. per 100 cm^3 soil found in the outdoor microplot test (five replications).

Variety	*Paratrichodorus*/100 cm^3 soil[z]	
Floralawn	8	A
Floratam	9	A
Seville	10	A
Roselawn	11	A
Floratine	17	A
Bitterblue	19	A
Common	25	B

[z] Means (five replications) followed by the same letters do not differ (P=0.05) according to the Waller multiple range test.

CHAPTER IV

LANCE NEMATODES ON ST. AUGUSTINEGRASS: EXTRACTION LOSSES FOR TWO SIZES OF SAMPLING UNIT AND CORE SIZE RECOVERY

Introduction

St. Augustinegrass [(Stenotaphrum secundatum (Walt.) Kuntze] lawns are a rapidly expanding Florida ecosystem characterized by high levels of energy input in forms such as irrigation, mowing, and fertilization (Busey and Burt, 1979). Hoplolaimus galeatus (Cobb 1913) Thorne, 1935 is frequently associated with unthrifty St. Augustinegrass in Florida. Because of the frequency of these problems, the lack of response to nematicides (Perry et al., 1970; Perry and Winchester, 1969) and increasing awareness of the vulnerability of Florida's aquifer to contamination by soluble pesticides, there is increasing need for information about how to best treat for or manage these nematodes.

Many approaches to managing pest populations, including the establishment of damage thresholds, require the ability to accurately and reliably quantify the pest's distribution, density, age structure and population dynamics. When the pest is a soilborne plant parasitic nematode, these estimates are dependent upon the efficiency and reliability of extraction procedures used to separate them from their environment (Goodell, 1982).

Separation of nematodes from soil is influenced by a multitude of factors. These include soil type (Ayala et al., 1963; Barker et al., 1969a; Harrison and Green, 1976), nematode species, time of year, extraction method used (Viglierchio and Schmitt, 1983b), and manner and duration of sample storage (Barker et al., 1969b).

In examining various soil-extraction techniques for survey purposes, Harrison and Green (1976) decided the sieving-centrifugal method was the most versatile for the expected variations in soil types. In addition to its versatility with varying soil types, the technique does not depend upon nematode motility, which can vary seasonally (Barker et al., 1969a). This is the extraction method currently used by the University of Florida Nematode Assay Lab. If reliable thresholds or management techniques are to be established for H. galeatus associated with St. Augustinegrass, the necessary population estimates for the state of Florida will probably be made by this lab and must use this method or a modification of it.

The sieving-centrifugal flotation method of soil nematode extraction is a multistep process containing many steps where losses may occur. If the major sources of processing losses could be identified, the process might be modified to reduce them, or a correction factor might be included in data analysis. Factors known to influence the number of nematodes recovered by this technique include the type of soil, and the type, amount and specific gravity of the solution used for extraction (Rodriguez-Kabana and King, 1975; Viglierchio and Yamashita, 1983). If nematode populations are to be quantified, these factors must either be controlled or accurately and reliably estimated so that correction factors may be used.

The objectives of this study were to determine extraction losses of _H_. _galeatus_ from single cores of two sizes of sampling units taken at three depths, compare the extraction losses of the two units, and to relate these extraction losses to known levels of infestation. These data were to be used to determine an optimal sampling strategy for _H_. _galeatus_ associated with St. Augustinegrass, which can then be used in management studies.

Materials and Methods

Sampling Design

The 'Floratine' variety St. Augustinegrass site studied was selected because it represented a worst-case situation in terms of processing and counting time. The site possessed a high mean density of _Hoplolaimus_ _galeatus_, comparatively fine textured soils, and a thin thatch layer that necessitated additional sieving of the samples to prevent the organic matter from clogging the sieves and centrifugation tubes.

The study area was divided into 10 strata based on visible surface criteria. Within strata, samples were taken randomly, their number dependent upon the surface area of the stratum. The design was, therefore, a stratified random one with proportional allocation on the basis of area (Cochran, 1977).

Each sample point was further stratified into three depths, 0-7.6 cm, 7.7-15.2 cm, and 15.3-22.9 cm. Two sampling unit sizes, a 10.2-cm-d core and a 2.2-cm-d core, were also examined. The smaller core was nested within the larger one. The total number of samples was, therefore, 43 sample points x three depths x 2 unit sizes, or 258.

Processing Procedure

All samples were bagged and processed separately by a modification of the sieving centrifugal flotation method described by Ayoub (1980). Because of the larger volume of the 10.2-cm-d sampling unit, some processing steps differed from those for the small sampling unit.

Samples taken with the 10.2-cm-d unit were dry sieved by passing the soil through stacked sieves (9.52-mm openings and hardware cloth, ca. 6-mm openings) to remove roots and foreign matter.

The 10.2-cm-d sample was washed through a kitchen sieve into a 19-L bucket and the water level brought to 9.5-L. The soil suspension was vigorously stirred until all the soil was suspended, then allowed to settle for 30 seconds before pouring the suspension into another bucket through a horizontally held 600-μm sieve. The particles of organic matter collected on the sieve were rinsed with a stream of collected on the sieve were rinsed with a stream of pressurized water directed through the sieve and into the bucket.

The contents of the second bucket were stirred until completely suspended and settled for 30 seconds. The mixture was passed through a 38-µm sieve held at a 40^0 angle. Sieve clogging was prevented by tapping its edge against the sink wall when necessary. The sieve contents were backwashed directly through a funnel into one or more 100-cm^3 plastic centrifuge tubes, depending upon the amount of material retained upon the sieve. The 0-7.6 cm depth required at least three tubes. The 38-µm sieve was then backwashed with a gentle stream of water to concentrate the remaining material, which was washed into the centrifuge tube, and the funnel given a final rinse into the tube.

The centrifuge tubes were adjusted with water to one cm from the top and centrifuged (R.C.F.=1244 G) in a swinging head centrifuge for three minutes. The supernatant was carefully poured out except for a layer capping the sediment.

The tubes were half filled with 454 g/L water-sucrose solution (Thistlethwayte and Riedel, 1969), mixed in bulk for this study to insure minimal density variation, and preserved with 8-quinolinol sulfate. The pellet was suspended by agitation with a metal spatula and solution adhering to the spatula was rinsed into the tube with more of the sugar solution. The tubes were filled uniformly and centrifuged

(R.C.F.=1244 G) for three minutes before the solution was poured through a 25-µm sieve held at a 40^0 angle.

The sieve contents were backwashed directly (McSorley and Parrado, 1981) into a labeled liquid scintillation vial through a funnel. The remaining material was concentrated by backwashing and rinsing the concentrate into the vial, followed by a rinse of the funnel.

The 2.2-cm-d units were treated similarly to the 10.2-cm-d units, except that the preliminary dry sieving and the 600-µm sieving were not performed. These steps were necessary for the large sample unit because of the extra number of roots and particulate organic matter associated with the greater volume.

The samples in the liquid scintillation vials were settled for seven hours and their fluid level adjusted to 5-ml by removing the surface water with a pipette and checking it to ensure no nematodes had been removed. The vials were heated in a 40 C water bath for 20 minutes before 5-ml of 10% formaldehyde solution of the same temperature was added and the vial agitated.

The sample was counted by pouring the contents of the scintillation vial and rinsate into a gridded 3.8-cm-d plastic plate. The nematodes were allowed to settle, and those adhering to the sides of the plate moved to the bottom. The entire plate was searched systematically with the aid of the grids using a stereo microscope. The bottom debris was carefully moved aside during counting to ensure all nematodes were seen.

All plant-parasitic nematodes were counted, but the numbers of four Hoplolaimus galeatus life stages [second stage juveniles (J2), third+fourth stage juveniles (J34), males and females] were tallied separately. The numbers of each of these life stages was summed to get the total number of lance nematodes in the sample (total).

Processing Checks

Five randomly selected sample points (i.e. three large and three small samples/point) were chosen for evaluation of extraction and counting efficiencies. Figure 4-1 outlines these check procedures. Six different processing steps were audited: three involved sieving steps (checks sieve 1, sieve 2, and sieve 3 of Figure 4-1), two examined steps designed to separate nematodes from the soil (soil and pellet checks) and the sixth checked the water supernatant commonly discarded (check water). Except for the soil and the pellet checks (Figure 4-1), which were processed through the remaining portion of the sieving-centrifugation procedure, material from the check points was collected and examined without further manipulation other than preservation.

Separate analyses were performed for the two sizes of sampling units. Linear comparisons for soil versus sieving losses and analysis of variance on transformed data, which were expressed as a percentage (Little, 1985) of the total nematode numbers or life stage counted in the final step, were used to determine the losses. Mean separation by the Waller-Duncan test was used to determine points of major extraction loss. Linear regression of total extraction losses upon the number counted in the final step was used to determine a potential correction factor. The question as to whether a particular sample unit had different total and component losses from the other was addressed with chi-square analysis using untransformed data. All analyses were done using the SAS (1982a) system.

Artificial Infestation-Recovery

While sampling, it was noticed that the two sampling units might not have recovered the entire soil volume possible. The soil level inside the 2.2-cm-d sampling unit was frequently lower than the surrounding ground level, occasionally as much as several cm lower. The 10.2-cm-d sampling unit seemed to lose some soil from the bottom of the core, though the amount varied with the soil moisture and the number and placement of roots inside the sampling unit. The exact nature and amounts of these losses, if truly present, could not be determined but may have been significant. For this reason the sampling process was incorporated into an artificial infestation and recovery test.

A stock population of *Hoplolaimus galeatus* grown on sorghum (*Sorghum bicolor* [L.] Moench) in a greenhouse was removed from the soil by centrifugal flotation (Ayoub, 1980).

Individual nematodes were hand transferred to create three different densities, 11, 22, and 42 lance nematodes per 100 cm^3 of soil. The nematodes were added to 3300 cm^3 of sieved soil (6 Cb moisture) containing St. Augustinegrass roots, and mixed by tumbling the mixture in a large plastic bag inflated with air.

The soil added to the bag was steam pasteurized while the roots used were obtained from 'Floralawn' variety St. Augustinegrass grown in 15.2-cm-d PVC microplots containing methyl bromide treated soil. For use, the stolons and nodes of the turf in the microplots were excised in a thin layer and saved, and the soil was excavated to a 14 cm depth and sieved to remove the roots. The microplot soil at this depth was leveled and compacted. The nematode-soil-root mixture was added to the microplots, making a 9.6 cm deep infested layer. The soil was lightly packed and the mat of turf stolons and nodes placed over it.

To give the nematodes more time to disperse, the microplots were sampled twenty-four hours later, using the same arrangement of the sample units described before. There were four replications of the low and intermediate nematode densities and three of the high density. All samples were extracted by the modified method described earlier. Linear regression of recovered nematodes upon those infested was used to determine the relationship of infestation level to recovery.

Results

Processing Checks

For both sizes of sampling unit, soil losses were greater than sieve losses, as shown by linear contrasts of sieving losses (sieve 2 and sieve 3 of Figure 4-1) versus the soil manipulation steps (checks soil and pellet). For the 2.2-cm-d sampling unit, only losses of C. annulatum at the 0-7.6 cm depth differed (P=0.007). For the 10.2-cm-d sampling unit, differences were observed for all H. galeatus life stages and for C. annulatum at all three depths and for H. dihystera at the 0-7.6 cm depth.

Calculated losses for the six processing checks, as a percentage of the number counted in the final step, are presented in Table 4-1 for lance nematodes and Table 4-2 for H. dihystera and C. annulatum. The points of major losses indicated by these checks (Table 4-1, 4-2 see Figure 4-1) differed somewhat between the two types of sampling units.

The major sources of loss for the 10.2-cm-d sample unit were those steps where nematode recovery depended upon their separation from the soil suspension. These losses were particularly apparent in the pellet remaining after the sugar flotation step but significance was also observed in the number of some H. galeatus life stages in the settled soil at the bottom of the bucket (checks pellet and soil respectively in Figure 4-1). Pellet losses of C. annulatum were particularly pronounced. Losses in the pellet showed a trend to decrease with increasing depth for spiral nematodes and increase with depth for the lance nematodes (Tables 4-1, 4-2).

The major extraction loss of H. galeatus in the 2.2-cm-d sampling unit was in the water supernatant from the first centrifugation (check water of Figure 4-1). This was most apparent at the 0-7.6 cm depth but could also be seen at the 7.7-15.2 cm depth. Another noteworthy point of loss, but clearly significant for only C. annulatum at the 0-7.6 cm and 7.7-15.2 cm depths, was the pellet check (check pellet).

The chi-square test using raw recovery data of extraction losses summed over all six check steps was used to test the hypothesis that the proportional extraction losses of the two sampling units were the same. The data, presented by Table 4-3, did not support this hypothesis. Significant losses were observed at each of the three depths and when the data were examined over depths. There may have been a trend for the magnitude of these differences to diminish with depth. If so, an apparent increase in losses with depth by the 2.2-cm-d sample unit seemed to account for this observation. Otherwise, losses of the 10.2-cm-d unit were greater than those of the smaller.

When these processing losses were broken into the individual check steps, the only step which consistently showed significance with chi-square analysis was the pellet check. These differences also tended to diminish at the 15.2-23.0 depth.

To determine if reliable linear correction factors which could account for processing losses were appropriate, summed extraction losses were regressed upon the number counted in the final sample. Occasional significance but with low R^2 values (R^2 ranges of .001-.41) were observed. This indicates that these models explained a small portion of the variation in extraction losses.

Artificial Infestation-Recovery

Table 4-4 shows the percentage recovery of lance nematodes from the two sampling units in the artificial infestation and recovery trial. Percentage recovery was greater from the 10.2-cm-d sampling unit than the 2.2-cm-d sampling unit. Recovery of the small J2 life stage was less efficient than for the other, larger life stages.

To determine recovery relationships of the two differently sized sampling units, regressions of the number of lance nematodes recovered upon the number expected were performed. More model variation was explained for the 10.2-cm-d sample unit (R^2=.89, $P<0.0001$) than was explained by the 2.2-cm-d sample unit (R^2=.68, $P=0.001$).

Discussion

An effort was made to minimize extraction losses of the 10.2-cm-d sample unit by using a large volume of water to suspend the soil. Rodriguez-Kabana and King (1975) have shown that recovery from larger soil volumes increases with increasing dilution until no further change is noted. For this reason 9.5-L of water were used to suspend both samples. This same relationship probably applies to the relative suspension column inside the centrifuge tube.

Ayala et al. (1963) showed that when sample size was increased using their sugar flotation method, a plateau and eventual loss in the number of nematodes recovered resulted. The exact sample size where this plateau and decline occurred seemed to depend upon the soil type and nematode species.

In these results, such an occurrence may be evidenced by the pellet losses in the 10.2-cm-d sampling unit. The relative suspension column inside the centrifuge was probably less for the 10.2-cm-d samples since there were usually more sievings per tube than for the 2.2-cm-d unit, even though more centrifuge tubes were used.

Centrifuge tube losses might be reduced by using larger centrifuge tubes or an angled centrifuge head, as recommended by Harrison and Green (1976). They maintain that in this type of centrifuge head, soil particles are precipitated along the side of the tube before sliding to the bottom, thus trapping fewer nematodes.

Extraction may have been complicated by factors influencing the specific gravity of the sucrose solution. Criconemella xenoplax (Raski) Luc and Raski recovery, for instance, declines sharply when the specific gravity falls below about 1.160 (Viglierchio and Schmitt, 1983a). Further, Viglierchio and Schmitt (1983b) showed that the specific gravity of the sucrose solution may decrease, depending on the soil type and soil moisture to which it is exposed. Such complicated interactions could greatly influence the recoveries among depths. Perhaps such factors were operational in this study, accounting for the poor losses of C. annulatum on one hand and for the differential losses of spiral and lance nematodes with depths on the other. Certainly losses seemed to vary among depths and strata, and even within a stratum, depending upon the textural composition of the sample, so marked extraction variation probably existed.

Although the extraction losses for the 2.2-cm-d unit were high, and the losses for the 10.2-cm-d sampling unit were unacceptable, those for C. annulatum from the 10.2-cm-d sampling unit were distressing. It is obvious that some extraction factor or factors were not favorable for their recovery. The most reasonable explanation for this might relate to the recovery curves of Viglierchio and Schmitt (1983a) which show a sharp increase in percent recovery of C. xenoplax per increase of specific gravity.

Nematode losses in the water supernatant have been noted by others (see Harrison and Green, 1976). The addition of kaolin was recommended to help reduce these losses. The percentage losses in the water discard found in this study for the 2.2-cm-d sampling unit agree with those of

Viglierchio and Schmitt (1983b). Lower losses for the 10.2-cm-d unit may be due to increased sedimentation of the nematodes in the reduced suspension column.

Variation in extraction efficiency with soil type has been noted (Ayala et al., 1963; Harrison and Green, 1976; Viglierchio and Schmitt, 1983b) and discussed in relation to sampling (Goodell, 1982) elsewhere. Variable extraction factors may account for the poor relationship between summed extraction losses and the numbers counted in the sample. In any instance, numerous and time consuming checks seem to be necessary if reliable correction factors for _H_. _galeatus_ and other nematodes commonly associated with St. Augustinegrass are to be obtained. Such correction factors are likely to be large, a practice of questionable value in quantitative studies (Viglierchio and Schmitt, 1983b). This problem may be worse in urban than in agricultural situations since construction and excavation activities often create complex soil mosaics while thatch and organic matter may vary seasonally. Given the current extraction techniques, such calibrations for _H_. _galeatus_ and other nematodes commonly associated with St. Augustinegrass may be more expensive than their worth compared to taking additional samples, unless a longitudinal study of a particular population's demographics is planned.

The results of the artificial infestation-recovery trial are somewhat puzzling. The large sample unit showed much greater extraction losses than did the small unit, in basic agreement with Harrison and Green (1976) and Ayala et al. (1963). In the artificial infestation-recovery experiment the large sample unit had a greater percentage recovery, especially for the smaller life stages, and explained more of the model variation.

It is possible that the results of this trial reflect more on the mixing process rather than recovery. If the nematodes were not completely mixed into the soil, they would have been concentrated in small pockets. In this instance a large sample unit would capture more of these clumps than would the small unit--which would encounter fewer clumps and recover more zero observations than expected. Certainly more zero captures than predicted did occur for the small sampling unit. It is doubtful if the 24 hour dispersal time was long enough for the nematodes to have moved far from these pockets, even if they were not stunned or killed by the mixing.

Artificial infestation-recovery trials using Heterodera cysts were criticized by Green and Parrott (1966) because the cysts did not become incorporated into the soil aggregates or stuck to the soil particles and therefore resulted in unduly high nematode recoveries. A similar criticism might also apply to this study, especially since the soil had been previously sieved.

Overall, these results agree with those of Viglierchio and Schmitt (1983b) which indicate that there is room for improving the centrifugal-flotation process and that the current losses are perhaps the largest obstacle to quantitative and demographic studies of nematodes.

Table 4-1. Percent loss of *H. galeatus* by life stage and depth from six different centrifugal flotation steps expressed as a percentage of those counted in the final step.

Three depths (cm) within each *H. galeatus* lifestage[z]

Extraction Step[y]	Total			J2			J34			Male			Female		
	7.6	15.2	23.0	7.6	15.2	23.0	7.6	15.2	23.0	7.6	15.2	23.0	7.6	15.2	23.0
2.2-cm-d sample unit															
Water	9.0 A	3.2 A	6.2 A	0.0 A	0.0 A	0.0 A	4.0 A	2.0 A	8.4 A	1.3 A	0.0 A	0.0 A	4.0 A	2.9 A	3.1 A
Pellet	.16B	0.4 B	5.0 A	0.0 A	0.0 A	2.0 A	0.0 A	0.4 AB	6.8 A	0.4 AB	0.0 A	0.0 A	0.4 A	0.0 A	1.0 AB
Soil	0.8 B	0.0 B	0.1 A	0.0 A	0.0 A	0.0 A	1.3 A	0.0 B	0.2 A	0.0 B	0.0 A	0.0 A	0.0 A	0.0 A	0.0 B
Sieve 1	0.3 B	0.0 B	0.19A	0.0 A	0.0 A	0.0 A	1.0 A	0.0 B	0.4 A	0.0 B	0.0 A	0.0 A	0.0 A	0.0 A	0.0 B
Sieve 2	0.0 B	0.5 B	0.0 A	0.0 A	4.0 A	0.0 A	0.0 A	0.0 B	0.0 A	0.0 B	0.0 A	0.0 A	0.0 A	0.0 A	0.0 B
Sieve 3	0.0 B	0.1 B	0.0 A	0.0 A	2.0 A	0.0 A	0.0 A	0.0 B	0.0 A	0.0 B	0.0 A	0.0 A	0.0 A	0.0 A	0.0 B
10.2-cm-d sample unit															
Water	0.0 C	0.2 B	0.7 B	0.0 A	0.0 A	0.4 B	0.4 B	0.4 B	0.3 B	0.0 C	0.0 C	0.3 B	0.0 C	0.0 B	0.1 B
Pellet	110.0 A	129.0 A	680.0 A	1.0 A	9.0 A	77.4 A	121.0 A	74.0 A	180.0 A	31.4 A	19.3 A	121.0 A	150.0 A	164.0 A	380.0 A
Soil	16.2 B	8.8 B	10.8 B	0.0 A	7.8 A	0.7 B	2.0 B	8.7 B	15.0 B	18.5 AB	8.5 AB	12.7 B	16.0 B	8.7 B	11.4 B
Sieve 1	0.0 C	0.0 B	0.0 B	0.0 A	0.0 A	0.0 B	0.0 B	0.0 B	0.0 B	0.0 C	0.0 C	0.0 B	0.0 C	0.0 B	0.0 B
Sieve 2	2.5 C	1.2 B	2.3 B	0.0 A	1.2 A	5.5 B	1.2 B	1.7 B	0.9 B	2.6 BC	1.4 BC	0.1 B	2.5 C	0.5 B	0.7 B
Sieve 3	0.0 C	0.6 B	0.8 B	1.3 A	7.8 A	4.0 B	0.0 B	0.9 B	0.3 B	0.0 C	0.0 C	0.0 B	0.0 C	0.0 B	0.0 B

[y] The exact placement of the six processing checks is better visualized by referring to Figure 4-1.

[z] Means in the same column followed by the same letter do not differ significantly at the $P \leq 0.05$ as judged by the Waller-Duncan test.

Table 4-2. Percent recovery of *H. dihystera* and *C. annulatum* by depth and sample unit from six centrifugal flotation extraction steps, expressed as a percentage of those counted in the final step. Means of these percentages are given for two sampling units and three depths.

Extraction Steps[Y]	Three depths (cm) within each nematode species					
	H. dihystera[Z]			*C. annulatum*		
	7.6	15.2	23.0	7.6	15.2	23.0
	2.2-cm-d sampling unit					
Water	0.7 B	0.0 B	0.0 A	0.1 B	0.0 B	0.0 A
Pellet	2.0 AB	0.2 B	0.2 A	88.4 A	139.0 A	3.2 A
Soil	0.7 B	0.7 B	0.0 A	0.1 B	0.1 A	1.1 A
Sieve 1	0.0 B	0.0 B	0.9 A	0.0 B	0.0 B	0.0 A
Sieve 2	3.3 A	1.0 B	1.8 A	0.8 B	0.1 A	0.0 A
Sieve 3	0.0 B	0.0 B	0.0 A	0.0 B	0.0 B	0.0 A
	10.2-cm-d sampling unit					
Water	0.0 B	0.2 B	0.0 B	0.0 B	0.1 B	0.0 B
Pellet	1464.0 A	1714.0 A	70.6 A	13366.0 A	11682.0 A	10240.0 A
Soil	19.0 B	7.3 B	2.3 B	19.0 B	9.5 B	12.5 B
Sieve 1	0.0 B	0.0 B	0.0 B	0.0 B	0.0 B	0.0 B
Sieve 2	8.1 B	0.9 B	4.9 B	8.1 B	5.4 B	5.4 B
Sieve 3	0.0 B	0.0 B	0.0 B	0.0 B	0.0 B	0.1 B

[Y] The exact placement of the six processing checks is better visualized by referring to Figure 4-1.

[Z] Means in the same column followed by the same letter do not differ significantly at the $P \leq 0.05$ as judged by the Waller-Duncan test.

Table 4-3. Chi-square analysis of summed recovery losses of the processing checks. Means of losses from each unit for the three depths and chi-square statistic.

	Mean number of nematodes lost			
	Mean value for each depth (cm)			
Nematode	0-7.6	7.7-15.2	15.3-23.0	0-23.0
	2.2-cm-d sampling unit			
H. galeatus				
J2	1.4	1.7	2.0	3.6
J34	1.8	1.7	2.9	3.8
Male	1.6	1.4	1.7	3.6
Female	1.8	1.7	2.2	3.8
Total	2.4	2.0	3.0	4.0
H. dihystera	2.6	1.9	2.2	3.8
C. annulatum	3.3	3.4	2.2	6.3
	10.2-cm-d sampling unit			
H. galeatus				
J2	1.8	3.3	4.5	4.6
J34	3.8	3.9	8.1	10.4
Male	3.5	3.2	4.6	5.4
Female	5.4	5.1	9.8	11.8
Total	8.7	9.4	20.7	27.2
H. dihystera	21.5	9.5	3.6	38.0
C. annulatum	59.7	56.6	54.7	175.8
	Chi-square statistics[z]			
H. galeatus				
J2	16.4**	41.8	34.2*	223.7***
J34	48.7	55.4*	86.8	472.7***
Male	29.2***	35.0*	12.9	123.2**
Female	97.0***	71.0	117.3***	621.1***
Total	157.9***	156.8**	191.0*	1206.6***
H. dihystera	183.3***	130.3***	56.6***	860.6**
C. annulatum	211.6***	175.0***	112.0***	64.1**

[z] Chi-square statistics followed by *, **, or *** are significant at the $P \leq 0.05$, 0.01, or 0.001 levels, respectively.

Table 4-4. Percent recoveries of *H. galeatus* by two differently sized sampling units from three known densities.

H. galeatus density/100 cm^3	Sampling unit (cm-d)	*H. galeatus* life stage means[z] J2	J34	Male	Female	Total
11.0	2.2	0	21	0	22	16
	10.2	78	82	86	54	73
22.0	2.2	0	23	26	14	48
	10.2	19	91	83	76	84
42.0	2.2	0	16	32	55	35
	10.2	20	94	73	53	74

[z] Means based on four replications at the low and intermediate densities and three replications at the high density.

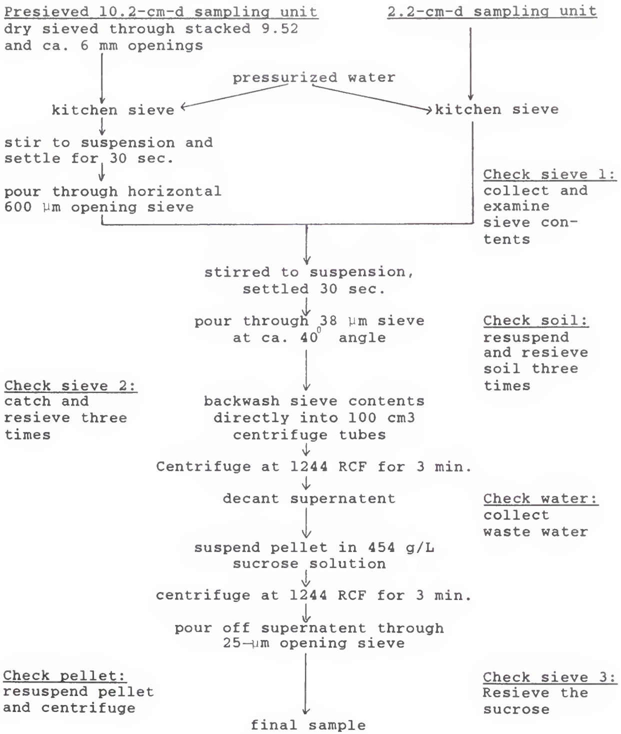
Presieved 10.2-cm-d sampling unit
dry sieved through stacked 9.52 and ca. 6 mm openings
2.2-cm-d sampling unit
pressurized water
kitchen sieve
kitchen sieve
stir to suspension and settle for 30 sec.
pour through horizontal 600 µm opening sieve
Check sieve 1:
collect and examine sieve contents
stirred to suspension, settled 30 sec.
pour through 38 µm sieve at ca. 40° angle
Check soil:
resuspend and resieve soil three times
Check sieve 2:
catch and resieve three times
backwash sieve contents directly into 100 cm3 centrifuge tubes
Centrifuge at 1244 RCF for 3 min.
decant supernatent
Check water:
collect waste water
suspend pellet in 454 g/L sucrose solution
centrifuge at 1244 RCF for 3 min.
pour off supernatent through 25-µm opening sieve
Check pellet:
resuspend pellet and centrifuge
Check sieve 3:
Resieve the sucrose
final sample

Figure 4-1. Schematic of the processing steps and check points.

CHAPTER V
LANCE NEMATODES ON ST. AUGUSTINEGRASS: SAMPLING PLANS

Introduction

In crop protection, the relationship between crop yield (Ferris, 1984A) and/or quality to the numbers of a pest is often desired. To ascertain this relationship, sampling is necessary. In order to obtain measurable and acceptable levels of accuracy and reliability (Goodell, 1982; Ruesink, 1980), samples should be taken in accordance with the target organism's biology and ecology.

In the case of soil-borne plant parasitic nematodes, sampling error is often compounded by subsampling (Francl, 1986) and extraction errors (Goodell, 1982). The extraction losses for separating Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935 from soil associated with St. Augustinegrass [Stenotaphrum secundatum (Walt.) Kuntze] have been described elsewhere (Chapter IV).

It is advisable to conduct a preliminary sample survey to determine the sampling design, the expected variability, practicality, and the costs (Goodell, 1982; Southwood, 1978). Such a design should consider the value and advisability of stratifying the area (Southwood, 1978) and incorporation of several differently-sized sampling units (Andrassy, 1962;

Southwood, 1978). One unit should be at the smallest size that can effectively sample the habitat, for it may allow a higher level of reproducibility for the same cost as taking fewer, larger samples (Southwood, 1978). Sampling larger units of a habitat is less likely to produce observations with zero or few counts than sampling with small units. Therefore, fewer larger sampling units than smaller ones might be required to estimate a population with a given reliability (Southwood, 1978).

The objectives of this study were to compare two differently-sized sampling units, determine the efficiency of horizontal stratification on the basis of visible surface criteria, examine the vertical distribution of *H. galeatus*, determine the amount of processing and counting time for the two sampling units, and relate these data to determine optimal sampling plans for *H. galeatus* and several other nematodes commonly associated with St. Augustinegrass. These data were to be used to develop sampling strategies which can be used in management studies of *H. galeatus* associated with St. Augustinegrass.

Materials and Methods

Preliminary Approximation of Sample Size

In order to obtain the best approximation of sample size possible from the major study, a preliminary approximation was made. Six St. Augustinegrass areas known to contain densities of *Hoplolaimus galeatus* ranging from 30-50/100 cm3 were sampled with single, 2.2-cm-d x 27 cm cores. These data were used to find the number of samples required to estimate the population mean with a reliability of 20%, assuming either a Poisson distribution or a negative binomial distribution (Karandinos, 1976). Combined data from all sites produced a worst-case scenario of n=43 while assuming a Poisson distribution. This sample size approximation provided the basis for the number of samples taken in the design study.

Sampling Design

A previously uninvestigated 'Floratine' St. Augustinegrass site was selected for intensive study because in terms of processing and counting time it represented a worst-case situation. The site possessed a high mean density of *H. galeatus*, comparatively fine textured soils and a thatch layer that would necessitate additional sieving of the samples to prevent the organic matter from clogging the sieves and centrifugation tubes.

The study area was divided into 10 strata based on visible surface criteria such as topography, type and density of weeds, and turf appearance and density. Within strata, samples were taken randomly, their number dependent upon the surface area of the stratum. The design was, therefore, a stratified random one with proportional allocation on the basis of area (Cochran, 1977). The number of samples per stratum ranged from two through six with a mode of four.

Each sample point was further divided into three depths, 0-7.6 cm, 7.7-15.2 cm, and 15.3-23.0 cm. Two sampling unit sizes, a 10.2-cm-d core and a 2.2-cm-d core, were also examined. The smaller core was nested within the larger one. The total number of samples was, therefore, 43 sample points x three depths x 2 unit sizes, or 258.

The objective of stratification is to minimize variations within the strata while maximizing the differences between them (Cochran, 1977). Analysis of variance using transformed data was used to determine the variation among strata. The gain due to stratification can be estimated by comparing the variance of the population mean per unit obtained from stratified random sampling to that which might have been obtained if the area had been randomly sampled. Comparison of these estimates in ratio form is termed the design effect and is calculated as follows (Cochran, 1977):

$$\text{Design effect} = \frac{1/N^2 \sum \frac{N_H \quad (N_H - n)}{n_H} \; s_H{}^2}{\frac{(N - n)}{nN} \; s^2}$$

Where:

N = possible number of samples.

N_H = number of samples possible within a given stratum.

n = actual number of samples taken.

s^2 = overall variance of the site.

$s_H{}^2$ = variance within a given stratum.

Extraction and Counting Methodology

The nematodes were extracted from the soil by a modification (Chapter IV) of the centrifugal-flotation technique (Ayoub, 1980). Because of the larger volume of the 10.2-cm-d sampling unit, some processing steps differed from the small sample unit.

Samples taken with the 10.2-cm-d unit were dry sieved by passing the soil through stacked sieves (9.52-mm openings and hardware cloth, ca. 6-mm openings) to remove some roots and foreign matter. Nematodes were not extracted from the roots because earlier test samples found none in them.

The sample was preserved in 5% formaldehyde and counted in a gridded 3.8-cm-d plastic plate. The nematodes were allowed to settle, and those adhering to the sides of the plate moved to the bottom. The entire plate was searched systematically with the aid of the grids using a stereo microscope. The bottom debris was moved aside during counting to insure all nematodes were seen.

All plant parasitic nematodes were counted, but the numbers of four *H. galeatus* life stages [second stage juveniles (J2), third and fourth stage juveniles (J34), males, and females] were tallied separately. The numbers of each of these life stages was summed to get the total number of lance nematodes in the sample (total). The totals of several other plant parasitic nematode genera commonly associated with St. Augustinegrass are also presented here.

Processing costs

The amount of sampling and processing time is related to the cost of the sampling scheme. Costs must be known in order to optimize the scheme (Cochran, 1977; McSorley and Parrado, 1982b). In research, time may be equated to cost. For each sample, every step of the entire sampling and processing procedure was timed.

Analyses were performed on the Statistical Analysis System (SAS Institute, Inc., 1982a, b, c). An attempt has been made to conform to the terminology defined by Karandinos (1976) and Ruesink (1980).

Results

Nematode density varied significantly among both strata and depths for both sampling units. The means at each depth and the analysis of variance for the different *H. galeatus* life stages as well as *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 and *Criconemella annulatum* (Cobb and Taylor, 1936) are presented in Table 5-1 for the 2.2-cm-d sampling unit and in Table 5-2 for the 10.2-cm-d sampling unit. Fewer *H. galeatus* life stages were recovered from the 0-7.6 cm depth in all instances. The 2.2-cm-d sampling unit did not differentiate between densities at the two deeper depths whereas the 10.2-cm-d unit usually did. Partially for these reasons, further analyses will be presented both by and over depths.

Most frequently, five of the 10 strata showed statistically distinct densities. The means of lance nematode totals in the strata ranged from 5 per 100 cm^3 soil to 79 per 100 cm^3 soil. These strata differences were judged great enough that Taylor's power law (Taylor, 1961) could be used to obtain preliminary estimates of population parameters within and across depths.

For each nematode variable, the variances from the 10 strata were regressed against the means of the strata to determine the dependence of the variance upon the mean. The relationship between the variance and the mean, as described by Taylor's power law (Taylor, 1961) was used to determine the appropriate normalizing transformation. A check on the efficacy of these transformations, by regression of the variances against the means using transformed data, was performed to determine residual dependency. Data conforming to the normal distribution are necessary for parametric statistical analyses.

For each sampling unit and depth, Table 5-3 shows the values of Taylor's power law and the model R^2 for both untransformed and transformed data. With the exception of the α values for Helicotylenchus dihystera and Criconemella annulatum at the 0-7.6 cm depth, no α value differed significantly from zero.

Except for the spiral nematodes (mostly Helicotylenchus dihystera but possibly minor numbers of an unidentified

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species), transformation of the variables obtained from the 10.2-cm-d sampling unit was effective. Some residual dependency was noted in variables obtained from the 2.2-cm-d sampling unit. These included the *H. galeatus* J2 and female life stages, as well as the spiral nematodes.

The regression slopes of each lance nematode life stage and both sample units were tested for their similarity to each other by creating a dummy variable (SAS Institute Inc., 1985c). All slopes and α values were found to be similar except for that belonging to the J2 life stage recovered by the 2.2-cm-d sample unit. For this J2 variable a Pr>F 0.02 was found for the model.

Stratification

The efficiency of stratification as determined by the design effect is presented in Table 5-4. Generally, a moderate reduction of the sampling variance was achieved (reductions of ca. 0 to 50%) as compared to simple random sampling.

Sample Unit Relationships

Chi-square contingency analysis was performed on untransformed data to determine if the proportions of the nematodes captured by the two sampling units were the same.

Table 5-5 indicates that the capture by the sampling units differed, depending upon the variable examined. For the lance nematode life stages, there seemed to be a tendency for the capture difference to decrease with increasing depth.

The total of the lance nematode life stages per unit recovered by each core size was not different for any depth. It is notable, however, that when depths were considered as a single unit (0-23 cm), differences were observed for the total lance nematode density as well as for each life stage. A situation similar to this is seen for the ring nematode, C. annulatum. Figures 5-1 and 5-2 present two frequency plots illustrating the differences in the distribution of total lance nematodes numbers recovered by the two sample units. In all instances, the smaller sampling unit had greater frequencies of zero and low numbers than did the large sampling unit, which also possessed a longer right-hand tail.

Processing Costs

The amount of time required to process and count a soil sample is often the limiting factor determining the number of samples which can reasonably be taken. Many of the major steps in the sampling and extraction process and the times required to complete them are shown in Table 5-6 for both sample units. The time to collect the sample is given as the removal time for that layer only; the removal time of the upper layers is not included. With a 20 cm long unit, removal of the upper layers would not double the presented sampling times, and would make little difference in the final analyses because of their small contribution relative to other processing and counting components.

Except for the time to actually collect the sample, times for the 10.2-cm-d unit were greater than those for the 2.2-cm-d sample unit. This difference was especially evident for the time required to count the samples. The time necessary to process samples varied significantly among the strata (Table 5-7). Topographically lower lying strata and the 0-7.6 cm depth required more processing time.

Determination of the Sampling Plans

The time necessary to process samples varied significantly among the strata. If collecting, processing, and counting time is treated as the cost of the sampling (Goodell and Ferris, 1981; McSorley and Parrado, 1982b), these differences mean that the cost per unit is not the same in all strata. Accordingly, when sample size is determined, the costs cannot be considered equal as assumed by Neyman's allocation (Cochran, 1977), but must be weighted in relation to the stratum variance. Sample size was determined as follows (Cochran, 1977):

$$n = \frac{(\Sigma W_H \; S_H \sqrt{C_H}) \quad \Sigma W_H \; S_H / \sqrt{C_H}}{V + (1/N) \; \Sigma W_H \; S_H{}^2}$$

Where:

N = number of potential samples in the site.

N_H = number of potential samples in the stratum.

W_H = number of potential samples in the stratum divided by $N(N_H/N)$.

S_H^2 = the estimation of the population standard deviation within the stratum.

c_H = cost of the sampling (in time) within the stratum.

Table 5-8 presents the number of samples necessary to estimate the population mean $(\bar{x} \pm \alpha_{.05}\sqrt{s})$ (Cochran, 1977) at different levels of confidence for both sample units and for depths. Data for these calculations is unadjusted for extraction losses, because of the poor predictability of these models (Chapter IV).

The same data are presented in Table 5-9 but depict the amount of time that would be required to take and process the samples. These data were uncorrected for the capture efficiency, as determined by the artificial infestation/sampling study.

Discussion

The α and β values of Taylor's power law provide some significant information about the organism. The β value is taken as an index of aggregation (Taylor, 1961), which is free of underlying assumptions about the distribution of the population. It is also a characteristic of the species, at least of a given nematode species in a particular soil type (Boag and Topham, 1984). The intercept α is both a sampling factor and a variate with the environment the species occupies (Taylor et al., 1983).

That neither the α nor the β values of Taylor's power law for Hoplolaimus galeatus life stages differed from one another (Table 5-3), even between the two sample units and depths, seems to indicate that within the limits of this study, the life stages exhibit similar aggregation and gross environmental preferences. Lack of differences for β values between depths has previously been observed for other nematodes (Boag and Topham, 1984) and these data concur. These data also may be interpreted as preliminary evidence that Taylor's power law may provide a robust and useful tool for working with Hoplolaimus galeatus in different St. Augustinegrass situations.

Animals with β values of two or greater are said to be highly aggregated (Taylor, 1961). The β values obtained from Taylor's power law indicate that most of the nematodes observed in this study exhibit only moderate degrees of aggregation. This agrees with McSorley et al. (1985) and Boag and Topham (1984) who found similar β values for other soil nematodes. These data also indicate that a check of the transformed data for residual dependency of the variance upon the mean is a wise precaution when parametric analyses are to be used.

Stratification was shown by Goodell and Ferris (1981) to be an effective sampling strategy. In this instance, however, previous knowledge of the nematode distribution and soil type existed. These data indicate that the surface characteristics of St. Augustinegrass sites can be used to sample unknown lance nematode populations by establishing effective strata. If an area is to be intensively studied (e.g. 20% confidence level), a more effective procedure might be to presample the area in a pattern described by McSorley et al. (1985), and determine the best stratification, sample allocation within the strata and the total number of samples required to meet the study objectives. Such a procedure would have to be executed fairly rapidly to allow as small a change in the target population as possible. It is also important that the additional presampling or stratification costs do not eliminate the gains due to stratification.

In this study, fewer samples would have been required to estimate the total lance nematode population at the 15.3-23.0 cm depth than at the shallower depths since their density was greater. This was because of the higher densities, lower variances, and the faster processing times associated with the deeper depths. Depending upon the objectives of a given study, and if this kind of vertical distribution and relationship between the density at this depth and all other depths holds constant throughout the year, it might be best to concentrate the sampling on this depth.

Without considering extraction losses, the sampling plans presented here indicate that fewer samples and less time will be required to estimate populations of organisms which are rare or perhaps difficult to extract (McSorley and

Parrado, 1981), as for the lance nematode J2 life stage in the shallower depths of this study, using a larger rather than a smaller sampling unit. More abundant organisms or larger life stages are usually more cheaply sampled by smaller sample units. The extraction losses, however, cannot be ignored and eliminate the 10.2-cm-d sampling unit from serious consideration until better and hopefully more rapid extraction techniques can be developed.

Table 5-1. Means and analysis of variance showing density differences for *Hoplolaimus galeatus* life stages, *Helicotylenchus dihystera*, and *Criconemella annulatum* for the 2.2-cm-d sampling unit among strata and depths using transformed data.

Nematode	Mean density			F-values[z] by effect		
	0-7.6	7.7-15.2	15.3-23.0	Strata	Depth	Strata x Depth
H. galeatus						
J2	0.4	1.0	1.1	2.6**	4.0*	0.8
J34	1.8	4.6	5.7	6.8***	10.4	0.4
Male	1.7	4.1	5.7	9.0***	12.4	1.6
Female	3.0	7.7	10.4	9.3***	18.2***	0.6
Total	7.0	17.4	23.0	8.5***	3.4***	0.4
H. dihystera	24.6	11.5	4.0	7.0***	7.5**	10.4
C. annulatum	22.2	25.8	29.4	4.3***	12.7***	2.3*

[z] F-values followed by *, **, or *** are significant at the $P \leq 0.05$, 0.01, or 0.001 levels, respectively.

Table 5-2. Means and analysis of variance showing density differences for *Hoplolaimus galeatus* life stages, *Helicotylenchus dihystera*, and *Criconemella annulatum* for the 10.2-cm-d sampling unit among strata and depths using transformed data.

Nematode	Mean density			F-values[z] by effect		
	0-7.6	7.7-15.2	15.3-23.0	Strata	Depth	Strata x Depth
H. galeatus						
J2	5.0	14.3	29.3	3.2**	10.1*	0.4
J34	18.6	35.4	64.5	11.6***	28.9***	0.7
Male	10.3	27.2	48.2	7.6***	30.8***	0.7
Female	18.0	50.3	90.8	9.6***	34.3***	0.7
Total	44.9	109.7	219.3	10.6***	35.7***	0.6
H. dihystera	221.8	12.8	47.3	14.0***	11.7***	0.6
C. annulatum	92.6	109.6	223.3	4.3***	12.7***	2.3*

[z] F-values followed by *, **, or *** are significant at the $P \leq 0.05$, 0.01 or 0.001 levels, respectively.

Table 5-3. Model R-squares and β values for log variance regressed on log means using raw and transformed data according to Taylor's power law, for two sample units and three depths.

	2.2-cm-d unit			10.2-cm-d unit		
	Model[z] R^2	B	R^2 trans-formed	Model[z] R^2	B	R^2 trans-formed
			0-7.6 cm			
H. galeatus						
J2	.99****	1.0	.99***	.08	2.3	--
J34	.43*	1.0	.0001	.59	1.2	--
Male	.95****	1.0	.0004	.83***	1.4	.13
Female	.75***	1.0	.001	.53**	1.3	.16
Total	.67**	1.5	.06	.43*	1.4	.07
H. dihystera	.96****	1.3	.98**	.98***	1.4	.97**
C. annulatum	.01	1.7	--	.01	0.2	--
			7.6-15.2 cm			
H. galeatus						
J2	.98****	1.0	.07	.74**	1.9	.03
J34	.73***	1.4	.02	.45*	1.7	.31
Male	.26**	1.1	.00	.78***	1.8	.05
Female	.63**	1.6	.28	.69**	1.7	.00
Total	.46**	1.7	.26	.61**	1.8	.04
H. dihystera	.91****	1.2	.88***	.97****	1.4	.96***
C. annulatum	.62**	2.4	.09	.12	0.0	--
			15.3-23.0 cm			
H. galeatus						
J2	.86****	1.5	.67**	.65**	1.5	.55**
J34	.40*	1.8	.02	.80***	2.6	.31
Male	.43*	1.4	.03	.62**	2.3	.07
Female	.55**	1.0	.61**	.72***	2.0	.03
Total	.56**	1.2	.72**	.64**	1.8	.00
H. dihystera	.96****	1.2	.98****	.95****	1.4	.94****
C. annulatum	.19	2.1	--	.19	2.1	--

[z] R-square value followed by *, **, ***, or **** are significant at the 0.05, 0.01, 0.001, or 0.0001 levels, respectively.

Table 5-4. Estimation of the gain due to stratification, by use of the design effect, for two sample units within and over depths.

Variable	2.2-cm-d unit depth				10.2-cm-d unit depth			
	7.6[z]	15.2	23.0	0-23.0	7.6	15.2	23.0	0-23.0
H. galeatus								
J2	.91	.79	.92	.92	.96	.73	.38	.79
J34	.88	.77	.84	.81	.71	.84	.36	.74
Male	.73	.78	.49	.76	.66	.70	.47	.84
Female	.69	.75	.27	.65	.71	.75	.47	.82
Total	.59	.72	.38	.67	.73	.77	.34	.79
H. dihystera	.97	.95	.82	.88	.75	.65	.85	.76
C. annulatum	.49	.78	.78	.76	.61	.89	.70	.92

[z] The design effect is the ratio of the stratified random sampling variance to the variance which would have been obtained from random sampling (Cochran, 1977):

$$\text{Design effect} = \frac{1/N^2 \sum \frac{N_H\ (N_H - n)}{n_H} s_H{}^2}{\frac{(N - n)}{nN}\ s^2}$$

Where:

N = possible number of samples.
N_H = number of samples possible within a given stratum.
n = actual number of samples taken.
S^2 = overall variance of the site.
$S_H{}^2$ =variance within a given stratum.

Table 5-5. Chi-square contingency analysis of nematode recovery by the two types of sampling units, within and over depths.

Nematode	Mean value for each depth (cm)			
	0-7.6	7.7-15.2	15.3-23.0	0-23.0
		2.2-cm-d sampling unit		
H. galeatus				
J2	0.4	1.0	1.1	0.8
J34	1.8	4.6	5.7	4.0
Male	1.7	4.1	5.7	3.8
Female	3.0	7.7	10.4	7.0
Total	7.0	17.4	23.0	15.7
H. dihystera	24.6	11.5	4.0	13.8
C. annulatum	22.2	25.8	29.4	25.8
		10.2-cm-d sampling unit		
H. galeatus				
J2	5.0	14.3	29.3	16.1
J34	18.6	35.4	64.5	39.3
Male	10.3	27.2	48.2	28.4
Female	18.0	50.3	90.8	52.8
Total	44.9	109.7	219.3	124.6
H. dihystera	221.8	109.7	47.3	126.1
C. annulatum	92.6	132.4	223.3	148.9
		Chi-square statistics[z]		
H. galeatus				
J2	62.6***	164.1**	195.0	610.4****
J34	303.6**	407.5*	441.0	1726.7****
Male	266.2****	391.6*	467.1	1580.6****
Female	285.2**	639.9	729.8	2553.1****
Total	468.2	859.0	1000.0	3900.6**
H. dihystera	984.0****	560.3****	413.7****	3855.3****
C. annulatum	983.5	1130.9	1160.0	6184.2*

[z] Chi-square statistics followed by *, **, ***, or **** are significant at the $P \leq 0.05$, 0.01, 0.001, or 0.0001 levels, respectively.

Table 5-6. Time (seconds) for procedure by depth and sample unit size.

	Two sampling units, three depths					
	0-7.6 cm sample unit		7.7-15.2 cm sample unit		15.3-23.0 cm sample unit	
Step	2.2	10.2	2.2	10.2	2.2	10.2
Collect sample	39	29	41	26	43	26
Extract Roots	75	1125	74	747	48	571
Wash Sample	521	1305	514	1151	511	1084
Centrifuge	512	732	465	538	491	567
Count Sample	494	4093	430	2924	422	3127
Weigh Roots	74	129	56	95	57	93
Total ± Std. Dev.	1715 ± 702	7413 ± 2056	1580 ± 690	5481 ± 1517	1572 ± 693	5468 ± 2201

Table 5-7. Two-way analysis of variance of the amount of processing and counting time. Individual analysis for each sampling unit.

	2.2-cm-d sampling unit	
Source	D.F.	F-value[z]
Strata	8	10.8***
Depths	2	0.8
Strata * Depths	16	0.5
Error	49	
Total	75	
	10.2-cm-d sampling unit	
Source	D.F.	F-value
Strata	8	29.4****
Depths	2	19.1****
Strata * Depths	16	0.5
Error	49	
Total	75	

[z] F-values followed by *, **, ***, or **** differ at the $P \leq 0.05$, 0.01, 0.001, and 0.0001 levels, respectively.

Table 5-8. Number of samples required to estimate the population mean of the site with varying confidence levels, apart from a 1 in 20 chance ($\alpha = 0.05$). For two sampling units, within and over three depths.

	Number of samples							
Variable	2.2-cm-d unit depth				10.2-cm-d unit depth			
	7.6[z]	15.2	23.0	0-23.0	7.6	15.2	23.0	0-23.0
				.10 Confidence level				
H. galeatus								
J2	875	380	616	752	458	277	101	377
J34	456	294	353	492	224	181	76	244
Male	364	205	189	397	241	163	104	320
Female	300	222	83	258	189	163	95	304
Total	191	178	123	271	206	163	63	266
H. dihystera	120	615	1714	630	58	283	1075	428
C. annulatum	205	225	165	911	128	271	149	402
				.20 Confidence level				
H. galeatus								
J2	220	95	155	189	124	72	27	99
J34	115	74	89	123	58	47	19	63
Male	91	51	48	100	62	42	27	83
Female	75	56	21	65	49	42	24	79
Total	48	45	31	68	53	41	16	69
H. dihystera	30	157	433	160	15	78	318	117
C. annulatum	51	56	42	65	32	70	38	82
				.25 Confidence level				
H. galeatus								
J2	141	61	99	121	80	46	17	64
J34	73	47	57	79	37	30	13	40
Male	58	33	30	64	40	27	17	53
Female	48	36	13	42	31	27	15	51
Total	31	29	20	44	34	27	10	44
H. dihystera	20	101	277	102	10	51	208	76
C. annulatum	33	36	27	42	21	45	24	53

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[z] Rounded up to the nearest whole number.

Table 5-9. Time (in hours) necessary to process and count the number of samples necessary for three confidence levels. Within three depths and over depths for two sizes of sample units.

	Number of samples							
Variable	2.2-cm-d unit depth				10.2-cm-d unit depth			
	7.6	15.2	23.0	0-23.0	7.6	15.2	23.0	0-23.0
				.10 Confidence level				
H. galeatus								
J2	487.3	285.8	326.5	409.0	4435.7	439.4	166.7	645.4
J34	254.0	221.2	187.1	267.6	2169.4	287.1	119.6	417.7
Male	202.7	154.2	100.2	215.9	2334.0	258.5	163.6	547.8
Female	167.1	167.0	44.0	140.3	1830.4	258.5	149.4	520.4
Total	106.4	133.9	65.2	147.4	1995.1	258.5	99.1	455.4
H. dihystera	66.8	462.6	908.4	342.6	561.7	448.9	1691.0	732.7
C. annulatum	114.2	169.2	87.4	495.5	1239.7	429.8	234.4	688.2
				.20 Confidence level				
H. galeatus								
J2	122.5	71.5	82.2	102.8	1200.9	114.2	42.5	169.5
J34	64.0	55.7	47.2	66.9	561.7	74.5	29.9	107.8
Male	50.7	38.4	25.4	54.4	600.5	66.6	42.5	142.1
Female	41.8	42.1	11.1	35.4	474.6	66.6	37.8	135.2
Total	26.7	33.8	16.4	37.0	513.3	65.0	25.2	118.1
H. dihystera	16.7	118.9	229.5	87.0	145.3	123.7	500.2	200.3
C. annulatum	28.4	42.1	22.3	35.4	309.9	111.0	59.8	140.4
				.25 Confidence level				
H. galeatus								
J2	78.5	45.9	73.0	65.8	774.8	73.0	26.7	109.6
J34	40.6	35.4	35.4	43.0	358.3	47.6	20.4	68.5
Male	32.3	24.8	24.8	34.8	387.4	42.8	26.7	90.7
Female	26.7	27.1	27.1	22.8	300.2	42.8	23.6	87.3
Total	17.3	21.8	21.8	23.9	329.3	42.8	15.7	75.3
H. dihystera	11.1	76.0	76.0	55.5	96.8	80.9	327.2	130.1
C. annulatum	18.4	91.0	91.0	22.8	203.4	71.4	37.8	90.7

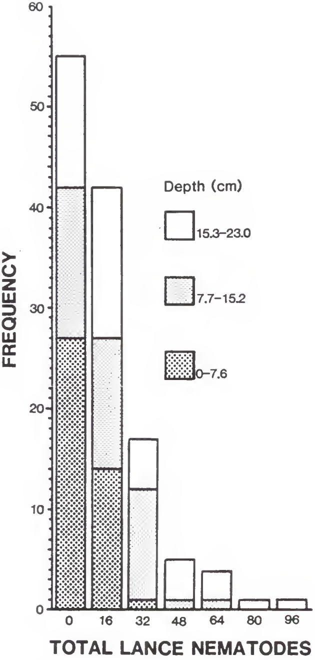

Figure 5-1. Frequency distribution of total _H. galeatus_ recovered by the 2.2-cm-d sampling unit for three depths.

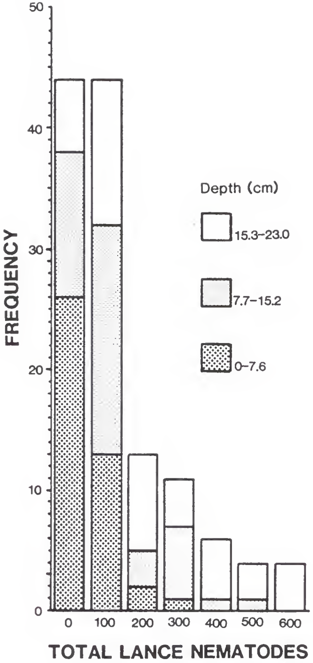

Figure 5-2. Frequency distribution of total _H. galeatus_ recovered by the 10.2-cm-d sampling unit for three depths.

CHAPTER VI

MONTHLY FLUCTUATIONS OF H. galeatus IN RELATION TO THE ENVIRONMENT AND SAMPLING

Introduction

Nematode advisory services are often asked two questions by growers who have submitted samples for analysis--are nematode kinds and numbers sufficient to be a problem; and will these nematode populations reach great enough numbers to cause a problem in the foreseeable future (Barker and Nusbaum, 1971)? Knowledge of temporal changes in nematode populations is essential to answer this latter question. The vertical distribution of a population may influence how and when it should be sampled. Both temporal and vertical population shifts could influence interpretation of assay results relative to the damage threshold and perhaps the nature and timing of a management practice.

Many factors may influence nematode population dynamics (Norton, 1979). Lance nematodes, Hoplolaimus spp., are commonly associated with unthrifty St. Augustinegrass lawns in Florida, and are known to be influenced by pH (Ahmad and Chen, 1980; Burns, 1971) soil texture, rainfall (Minton et al., 1960), and host plants (Ahmad and Chen, 1980). In advisory situations some of these data, such as soil texture, are probably unknown and costly to obtain. What are needed for both predictive and management purposes are easily obtained and inexpensive data which are related to population changes of the target population.

Climatological data might meet these criteria. While it is naive to expect a few simple climatic factors to explain population dynamics arising from complex edaphic and biological interactions, some explanatory power may exist in such data. The distribution of nematode genera in New Zealand pastures, for example, is better explained by climate than by soil texture (Yeates, 1981).

Rainfall is a climatic factor that is frequently examined because of its obvious force and importance to the plant host, its influence on soil moisture and nematode movement, and its ease of measurement. Hutton (1978) associated rainfall some weeks earlier to nematode populations but noted that the exact population response depended upon the soil texture. Both the number of days of rainfall and its accumulation were useful predictors when rainfall was moderate but flooding rains uniformly reduced populations. Rainfall and temperature data were combined into an index by Jones (1975) which he related to Trichodorus and Longidorus activity, even though the weather station was located many kilometers distant.

The objective of this study was to observe the vertical distribution and temporal fluctuations in both the age structure and density of lance nematode [Hoplolaimus galeatus

(Cobb, 1913) Thorne, 1935 populations associated with St. Augustinegrass, Stenotaphrum secundatum (Walt.) Kuntze. These data are to be used in designing effective sampling and advisory data.

Materials and Methods

Two St. Augustinegrass sites were sampled monthly from January 1985 through December 1985. On each sampling date, 51 samples were removed from each 16 m^2 site. There were 17 separate sample points per site with each point divided into three depths, 0-7.6 cm, 7.7-15.2 cm and 15.3-23.0 cm. Each sample was bagged and processed separately by a modification of the standard sieving-centrifugation method (Ayoub, 1980) detailed elsewhere (Chapter IV).

Nematodes associated with the roots were extracted for 24 hours using a modified Baermann method with Scotties^R tissues and non-macerated roots (see Endo, 1959). After extraction, the roots were dried at 60 C for two days, cooled in a desiccator, weighed, and their root length measured by counting the number of intersections on a 1-cm grid (Tennant, 1975). All nematodes in both the soil and root samples were counted, and four life stages of the lance nematodes were tallied separately: second stage juveniles (J2), third and fourth stage juveniles (J34), males and females and their sums (total). Lance nematodes were the predominant nematode in both sites. Criconemella spp., Paratrichodorus spp., Meloidogyne spp., and Xiphinema americanum Cobb occurred in low numbers throughout the year.

Site one was located approximately 32 km NW of Gainesville, Florida at the University of Florida Horticultural Unit. The 'Floralawn' variety was planted in a low lying area of Arredondo fine sand (ca. 93% sand, 5% silt, 2% clay) which occasionally remained soggy several days after frequent rains. The site received moderate maintenance and was cut at a height of about 8.0 cm with clippings returned to the soil. The site was covered by an automatic irrigation system and was not subjected to drought stress. The mean population density at the start of the study was 39.5 lance nematodes of all life stages per 100 cm^3 soil. Air temperatures and rainfall data were recorded daily near the site.

'Floratine' St. Augustinegrass was planted in the second site, which was located on a south facing slope on the Gainesville campus of the University of Florida. This site received some foot and occasional vehicular traffic. The maintenance level was low, primarily limited to close cutting (ca. 2.5 cm height). The soil was seemingly a mixture of material from construction activity. It possessed a hard pan at about 24 cm, which moved upward during dry periods at which times a rubber mallet was required to remove the 15.3-23.0 cm deep samples. The mean lance nematode population density at initiation of the study was 24.5 per 100 cm^3. Daily weather data and readings of photosynthetically active radiation were obtained from the university weather station, approximately 4 km from the site.

Data from the two sites were analyzed in both individual and combined form by analysis of variance. Locations, depths, and sample dates were used as main effects.

The age structure and correlations between preceding and succeeding life stages of the populations were examined among months. The age structure of a population is a strong determinant of future population density. A rapidly expanding population will contain a large percentage of young individuals whereas a declining population will have a large percentage of older individuals. Changes in the age structure of a population may indicate changes in conditions influencing it. Supportive age structure data would be useful as a gauge for developing management strategies as well as in basic research.

Correlations between the densities of various age groups would be useful for advisory purposes. Reliable correlations within months and among soil and root dwelling life stages would allow more rapid and economical population estimates for advisory programs or estimates for researchers.

Since nematode population densities change seasonally (Barker and Nusbaum, 1971), different sampling intensities may be required to measure them with the same reliability at different times of the year. An indication as to the periodicity of population oscillations would aid in prediction of sampling costs and in advisory circumstances where nematode samples may be taken as a precaution by pest control operators. The monthly means and variations of these study populations were used to determine the most cost effective periods of the year in which to sample (Snedecor and Cochran, 1967). The number of necessary samples may be estimated (Snedecor and Cochran, 1967):

$$n = \frac{s}{E\,\bar{x}}^{2}$$

Where:

n = the number of samples.
$\bar{x}$ = the mean of the population to be sampled.
s = the standard deviation of the population.
E = a predetermined standard error expressed as a percentage of the mean and held constant here, dropping it from the equation.

The formula was used to determine the number of samples required for each month of the study.

Multiple linear regression models were used to search for factors which might be useful in later studies. The independent variables examined here included temperature, rainfall and photosynthetically active radiation (PAR). Air temperatures were expressed as a maximum, an average, or were used as degree day accumulations on 10 or 20 C bases.

Rainfall was expressed as monthly accumulation or days of rain in the month. Both temperature and rainfall were combined by the index developed by Jones (1975). All variables and their combinations were examined with lag periods previous to the sampling date of one to two months. In all, 31 models were examined with a stepwise procedure (see Table 6-7).

Analyses were performed using the Statistical Analysis system (SAS Institute Inc., 1982a).

Results

Three major storm systems occurred in the Gainesville area in mid-August, flooding site one and making intensive sampling inaccurate. The August sample from this site was composed of a single bulked sample which resulted in missing comparison data.

The general population dynamics of lance nematodes in the two sites were somewhat different. Lance nematode numbers in site one were highest from May through July followed by September through October and were lowest in February and March (Figure 6-1). Populations in site two were highest from October through December followed by July and August and were lowest from March through May (Figure 6-2).

An overall analysis of variance incorporating sampling date, sites, and depths as main effects showed these factors to be significant for both soil and root dwelling *H. galeatus* life stages. Significant date by depth interactions were observed for all soil and several root-dwelling life stages (Table 6-1). To obtain better resolution of these effects, dates and depths were used as main effects with separate analyses for each site.

Tables 6-2 and 6-3 present these data for site one and site two, respectively, with the appropriate means. These data indicate that for both sites, the numbers of all soil life stages changed significantly during the study period and that there were different densities of the life stages among the three depths. A sample date by depth interaction was also apparent in these analyses, especially for site one.

To better define this interaction, a second analysis of variance was performed for each sample date, with sites and depths as main effects. The numbers of each life stage differed between the two sites on each sampling date except March. From May through October, greater numbers of lance nematodes were found in the upper 15.2 cm of soil than in the lower 15.3-23.0 cm. The J34 life stage was particularly prominent in the upper soil layers whereas the adults seemed to be more evenly distributed. In December a significant change in the vertical stratification of the nematodes occurred. More of them were found in the 7.7-23.0 cm depths than at the shallow depth (Figure 6-3). This change was more pronounced in site one than in site two.

Few lance nematodes were recovered from the roots from January to May and from November to December. The most common life stage recovered from the roots was the J34, by two to four times the next most common life stage. Correlations between any lance nematode variable and root length or dry root weights were low.

The percentages of each life stage for both sites and results of analyses of variance identical to that of Tables 6-2 and 6-3 are presented in Tables 6-4, 6-5, and 6-6. In site one (Table 6-4), the percentage of each life stage changed significantly ($P<0.0001$) during the study. Differences among the three depths were seen for all but the J2's, and significant ($P<0.01-0.0001$) sample date by depth interactions were observed, indicating that there were changes in the densities of these life stages among depths during the period.

Only the juveniles (J2 + J34) in site two (Table 6-5) showed significant changes in their percentages among sampling dates, and only the J34's showed differing densities among the depths. Only a slight ($P=0.05$) sampling date by depth interaction was observed for this variable.

Within a given sampling date, older life stages (J34 and beyond) and the sum of all soil dwelling life stages were significantly correlated with each other ($r=.64$ to $.99$), but these correlations were much lower with the J2's (ca. $r=.1$) and were often not significant. Very low correlations were observed between individual root-dwelling life stages and their counterparts in the soil (r=.00 to .21) whereas the sum of all root-dwelling life stages was correlated to the sum of those in the soil (r=.38 to .60), but was of variable significance.

Table 6-7 presents the relative sampling intensities which would have been necessary for each month of the study for both study sites. Fewest samples would have been required in July or in October and November.

Multiple linear regression models were better able to fit the total lance nematode population in site one (R^2=.82) than in site two (R^2=.49) (Table 6-8). The most important factors in site one were degree day accumulation with a 10 C base followed by rainfall accumulation delayed (lagged) by two months. For site two, the most important factor was twice lagged number of days of rain closely followed by photosynthetically active radiation (PAR). Degree day accumulation added little to the R^2 value and decreased the significance of the site two model. The single best model considering both sites included lagged degree day accumulations, PAR and two month lagged days of rain.

Discussion

Seasonal changes of lance nematode densities on bentgrass golf greens have been related to the maximal growth periods of this cool season turfgrass, lagged for a one or two month period (Lucas et al., 1978). A similar relationship between lance nematode numbers and growth periods of the warm season St. Augustinegrass seems to have occurred in this study.

In both sites the highest specific growth rates (Norton, 1979; specific growth rate = change in numbers/change in time*P_i) for H. galeatus occurred early in the season, possibly in response to the initiation of spring growth by their St. Augustinegrass host. The highest specific growth rates occurred in site one during March-May (4.3) and in site two from May-June (1.9) (see Table 6-5). During the summer months the specific growth rates ranged from .24 to .41.

Seasonal changes in the vertical distribution of lance nematodes have been noted not only in these study sites but in several other sites sampled. Intensive sampling of another site in January (Chapter V) found greatest densities at the 15.3-23.0 cm depth after an intense cold period whereas a preliminary screening of the same site one and one-half months previous to the cold period did not show such a vertical distribution. Therefore, density increases at the greater depths later in the year may be related to the onset of cold weather.

A population's age structure is important in determining future population densities and is an important indicator of favorable and unfavorable conditions. The largest percentage composition of females in the two populations was observed in May (Tables 6-4, 6-5, 6-6). It appears that this peak arose from slightly high third and fourth stage juvenile (J34) percentages present from February-April. The percentage of juveniles in both sites was also high from October through December. It seems that in both sites the J34's may have undergone a decrease in their rate of increase around September. Both sites showed visible decreases in their total numbers (Tables 6-2 and 6-3) during February.

These data might be interpreted to suggest that the best time to have tried to manipulate the future population growth of these study populations would have been around late January and continuing into April. This is also the time when fewest _H_. _galeatus_ are in the roots. These data lend some support to the possibility that heavy rains can detrimentally impact their populations, since reductions in the percentage of J34's seem to have occurred in August and September (Tables 6-4, 6-5, 6-6). The consistently greater percentage of adults than juveniles indicates that they are longer-lived.

Low correlations between younger life stages and older life stages the following months, coupled with simultaneous density changes between the various life stages, may indicate that lance nematodes passed through a generation between the monthly sampling periods.

Pest control operators or home owners collecting samples for nematode diagnosis during the cooler parts of the year should take care to insure that the sampling tool collects soil to the 23.0 cm depth. The number of samples they will need for the same degree of reliability will vary seasonally. For both locations, the number of required samples were greatest from February to June and were fewest in July and from October through December. Sampling will be examined further in a later chapter.

The best fitting multiple linear regression model for site one included one month lagged degree day accumulation and doubly lagged rain accumulation. The best model for site two included lagged PAR and double lagged number of days of rain.

In site one, the nematode population was associated with a host which had enough water and photosynthetic area (cutting height ca. 8 cm) to maintain regular functioning. In this instance, rain accumulation might impact more directly on the nematode populations since daily irrigations should remove many host and osmotic stresses. The apparent decline in the populations following the flooding August rains agrees with Hutton (1978).

In site two, the turf may have been limited by water availability since it was not irrigated, was situated on a south facing slope, and was underlain by a hardpan. It might be that significant PAR values in the model are related to both the reduced photosynthetic area of the closely cut turf and the decrease in photosynthetic activity resulting from water stress mechanisms initiated by the plant (see Peacock, 1981). Even slight rainfall might partially alleviate water stress, perhaps explaining the significance of the number of days of rain at this site and the population peak during the latter summer months when rains were more frequent. An apparent decline in the September population density might also be due to the heavy August rains.

Table 6-1. Overall analysis of variance of the two sites and three depths sampled monthly during 1985.

		H. galeatus lifestages in the soil[z]				
Source	D.F.	J2	J34	Male	Female	Total
Date	11	22.5****	25.4****	10.4****	17.3****	17.3****
Site	1	98.3****	25.1****	64.0****	121.3****	121.3****
Date * Site	11	20.3****	5.8****	12.4****	20.3****	20.3****
Depth	2	7.1****	41.2****	32.6****	16.1****	16.1****
Date * Depth	22	3.2****	1.7*	4.0****	3.2****	3.2****
Site * Depth	2	3.8*	0.7	17.7****	6.9****	7.0***
Date * Site * Depth	20	3.5****	5.0****	4.6****	3.2****	3.3****
Error	1097					
Total	1166					
		H. galeatus lifestages in the roots[z]				
Date	11					
Site	1	6.0****	8.6****	6.1****	8.9****	12.9****
Date * Site	11	10.0**	5.2*	24.7****	37.8****	24.1****
Depth	2	5.9****	4.6****	4.0****	6.4****	7.4****
Date * Depth	22	2.0	9.7****	4.0*	1.2	8.1***
Site * Depth	2	0.6	2.9****	1.3	0.6	2.3***
Date * Site * Depth	20	2.4	1.9	0.8	0.8	0.9
Error	1097	0.7	1.6	0.9	1.2	1.6*
Total	1166					

[z] F-values followed by *, **, *** or **** are significant at the $P \leq 0.05$, 0.01, 0.001 or 0.0001 levels respectively.

Table 6-2. Means of *Hoplolaimus galeatus* life stages in the soil for each sampling date and depth, and analysis of variance with sampling dates and depths as main effects. Site one.

	H. galeatus life stage				
Source[z]	J2	J34	Male	Female	Total
Date means					
	****	****	****	****	****
1	1.3	10.6	11.5	16.1	39.5
2	0.6	4.2	4.4	6.0	15.2
3	0.4	1.8	2.5	4.0	8.7
4	2.1	9.0	12.9	20.4	44.5
5	1.5	10.2	26.9	34.2	72.8
6	8.2	10.1	35.8	46.5	100.6
7	8.5	13.3	26.1	40.9	88.8
8	--	--	--	--	--
9	4.3	9.7	14.2	21.3	49.4
10	1.6	9.7	14.6	20.8	46.7
11	3.2	10.9	9.3	9.2	32.6
12	2.6	25.8	10.7	12.3	51.4
Depth (cm) means					
	**	****	****	****	****
0-7.6	3.4	13.7	23.3	25.1	65.5
7.7-15.2	3.7	10.5	14.6	23.2	52.1
15.3-23.0	2.3	7.2	7.7	14.7	32.0
Date * Depth	****	****	****	****	****

[z] Sources of variation within columns headed by **, ***, or **** are significant at the $P \leq 0.01$, 0.001, or 0.0001 levels, respectively, as determined by ANOVA with mean separation by the Waller-Duncan test.

Table 6-3. Means of _Hoplolaimus galeatus_ life stages in the soil for each sampling date and depth, and analysis of variance with sampling dates and depths as main effects. Site two.

	H. galeatus life stage				
Source[z]	J2	J34	Male	Female	Total
Date means					
	****	****	****	****	****
1	1.0	6.9	7.9	8.7	24.5
2	0.0	0.9	1.1	1.5	3.6
3	0.1	1.4	1.4	1.8	4.7
4	0.2	2.3	2.0	2.2	6.7
5	0.0	0.8	1.1	1.3	3.2
6	0.1	3.3	2.3	3.2	8.9
7	0.9	6.3	4.8	5.9	18.0
8	1.1	11.4	3.8	5.4	21.7
9	0.6	2.3	1.5	2.3	6.6
10	0.7	13.2	7.4	9.5	30.8
11	1.5	12.5	6.5	7.0	27.5
12	0.8	13.5	6.8	10.1	31.3
Depth (cm) means					
	**	****	***	***	****
0-7.6	0.8	9.4	5.2	6.4	21.2
7.7-15.2	0.6	5.8	4.7	5.8	17.5
15.3-23.0	0.4	3.6	3.0	4.0	11.0
Date * Depth		****			

[z] Sources of variation within columns headed by **, ***, or **** are significant at the $P \leq 0.01$, 0.001, or 0.0001 levels, respectively, as determined by ANOVA with means separation by the Waller-Duncan test.

Table 6-4. Means and analysis of variance with sampling dates and depths as main effects. Data for each life stage are expressed as a percentage of the summed soil and root dwelling lance nematode populations. Site one.

	H. galeatus life stage			
Source[Y]	J2	J34	Male	Female
Date means[Z]				
	****	****	****	****
1	2.2	15.0	13.1	21.7
2	1.8	23.6	13.2	32.1
3	1.4	18.7	13.7	23.0
4	5.6	20.8	24.8	48.7
5	2.3	16.6	32.5	48.6
6	12.8	14.8	26.4	46.0
7	10.7	17.2	25.4	46.6
8	--	--	--	--
9	7.2	19.8	28.4	44.5
10	3.6	23.1	30.0	43.2
11	11.0	34.7	26.6	27.7
12	4.7	47.7	23.4	24.2
Depth (cm) means				
		*	****	****
0-7.6	5.0	25.6	26.4	32.2
7.7-15.2	5.8	20.6	24.0	29.9
15.3-23.0	6.4	22.6	19.6	38.9
Dates * Depths	****	**	**	***

[Y] Degrees of freedom were Date = 11, Depth = 2, and Date * Depth = 22.

[Z] Sources of variation within columns headed by *, **, ***, or **** are significant at the $P \leq 0.05$, 0.01, 0.001, or 0.001 levels, respectively,as determined by ANOVA with means separation by the Waller-Duncan test.

Table 6-5. Means and analysis of variance with sampling dates and depths as main effects. Data for each life stage are expressed as a percentage of the summed soil and root dwelling lance nematode populations. Site two.

	H. galeatus life stage			
Source[Y]	J2	J34	Male	Female
Date means[Z]				
	***	****		
1	2.4	16.2	21.8	27.6
2	2.0	23.6	22.8	33.6
3	2.5	27.8	26.2	35.7
4	2.6	28.2	28.7	32.3
5	0.3	17.4	31.3	40.0
6	0.8	41.2	21.5	32.5
7	5.0	37.3	24.7	33.0
8	5.1	52.9	18.6	23.4
9	9.1	34.3	18.3	32.2
10	2.7	40.5	24.2	32.6
11	5.6	42.0	26.0	26.4
12	3.1	41.9	21.3	33.7
Depth (cm) means				
		***		
0-7.6	11.8	39.0	23.1	25.2
7.7-15.2	5.6	31.0	24.3	37.8
15.3-23.0	10.1	31.0	20.6	37.3
Dates * Depths		*		

[Y] Degrees of freedom were Date = 11, Depth = 2, and Date * Depth = 22.

[Z] Sources of variation within columns headed by *, **, ***, or **** are significant at the $P \leq 0.05$, 0.01, 0.001, or 0.001 levels, respectively, as determined by ANOVA with mean separation by the Waller-Duncan test.

Table 6-6. Percentage of each life stage (summed soil and root numbers) to total population (summed life stages in soil and roots) for 12 months over both sites.

	H. galeatus life stage					
Month	J2	J34	Male	Female	Juvenile	Adult
01	3.8	26.1	29.0	41.0	29.9	70.1
02	2.5	30.9	23.5	43.0	33.8	66.2
03	2.6	31.2	26.8	39.4	33.8	66.8
04	4.3	25.5	27.9	42.4	29.8	70.2
05	1.4	18.0	33.9	46.6	19.4	80.6
06	7.0	28.0	24.4	40.2	35.4	64.6
07	7.9	26.9	25.1	40.0	34.9	65.1
08	5.0	52.9	18.6	23.5	57.8	42.1
09	8.4	27.8	24.2	39.7	36.1	63.9
10	3.1	31.6	27.2	38.0	34.8	65.2
11	8.4	38.3	26.3	27.0	46.6	53.3
12	3.9	44.9	22.4	28.8	48.8	51.2

Table 6-7. Determination of monthly sample size for sites one and two.

H. galeatus life stage	Proportional number of samples[z]											
	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Soil	Site one											
J2	5	4	8	2	2	1	1	--	2	1	1	3
J34	3	3	6	1	1	1	1	--	2	1	1	2
Male	3	3	6	2	2	3	1	--	2	1	1	1
Female	3	2	4	1	1	2	1	--	1	1	1	1
Total	3	3	4	1	1	2	1	--	1	1	1	1
Root												
J2	26	52	--	--	--	3	11	--	19	52	13	--
J34	9	8	51	4	3	3	2	--	3	2	2	19
Male	14	--	25	7	6	2	4	--	4	3	3	25
Female	10	19	51	6	4	1	5	--	2	3	3	16
Total	10	9	20	3	2	2	2	--	2	2	2	14
Soil & Root	3	3	5	1	1	2	1	--	1	1	1	1
	Site two											
Soil												
J2	3	50	17	6	50	14	2	3	3	2	3	2
J34	3	2	2	4	4	1	1	2	1	1	2	1
Male	3	3	2	2	2	1	1	2	2	1	1	1
Female	2	2	2	1	1	1	1	1	1	1	1	1
Total	2	2	1	2	1	1	1	1	1	1	2	1
Root												
J2	--	--	--	50	--	--	25	25	--	16	--	52
J34	4	12	17	14	50	6	2	4	6	5	4	3
Male	15	--	--	--	--	25	3	7	--	50	9	11
Female	11	--	51	--	25	9	5	5	8	28	11	5
Total	5	12	12	11	16	4	1	4	4	5	3	5
Soil & Root	2	2	1	2	1	1	1	1	1	1	2	1

---

[z] Dropping E from equation:

$$n = {\frac{\text{standard deviation}}{\text{E x mean}}}^{2}$$

Table 6-8. Representative multiple linear regression models used in this trial, and their R^2 values in both sites, for all *H. galeatus*.

Model	Site 1[v]	Site 2
Max, rain accum.[w]	.21	.36
Max, rain accum., PAR[x]	.23	.38
Max, lag 1 rain accum., PAR	.41	.29
Max, lag 2 rain accum., PAR	.28	.30
Lag 1 max, lag 2 rqain accum., lag 1 PAR	.73**	.46
Max, days rain	.21	.41
Max, days rain, PAR	.23	.34
Max, lag 1 days rain, PAR	.25	.31
Max, lag 2 days rain, PAR	.30	.31
Lag 1 max, lag 2 days rain, lag 2 PAR	.76***	.46
Deg 10[y], rain accum.	.65**	.29
Deg 10, rain accum., PAR	.66**	.33
Deg 10, lag 1 rain accum., PAR	.65**	.24
Deg 10, lag 2 rain accum., PAR	.82**	.24
Lag 1 Deg 10, lag 2 rain accum., lag 1 PAR	.68**	.49*
Deg 10, days rain	.69**	.25
Deg 10, days rain, PAR	.10	.33
Deg 10, lag 1 rain, PAR	.69*	.22
Deg 10, lag 2 rain, PAR	.67*	.18
Lag 1 Deg 10, lag 2 rain, lag 1 PAR	.77**	.46
Effective temperature (EF)[z], rain accum.	N.S.	.36*
EF, PAR	N.S.	.38
Lag 1 EF	N.S.	N.S.
Lag 1 EF, PAR	N.S.	N.S.
Lag 2 EF	N.S.	N.S.
Lag 2 EF, lag 1 PAR	.58**	N.S.
EF, days of rain	N.S.	N.S.
EF, PAR	N.S.	.38*
Lag 1 EF, PAR	N.S.	N.S.
Lag 2 EF	N.S.	N.S.
Lag 2 EF, PAR	N.S.	N.S.

[v] R^2 values followed by *, **, or *** are significant at the $P \leq 0.05$, 0.01, and 0.001 levels respectively.

[w] Rain accum. = rain accumulation during the month.

[x] PAR = photosynthetically active radiation.

[y] Deg 10 = degree day accumulation on a 10 C base.

[z] as adapted from Jones (1975).

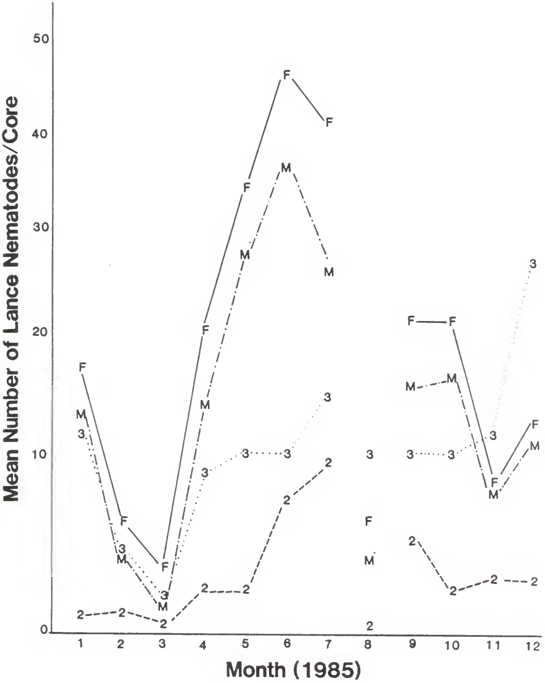

Figure 6-1. Number of four lance nematode life stages summed over three depths. Site one. The lines are not connected to sampling date eight to indicate that only one bulk sample was drawn due to flooding of this site.

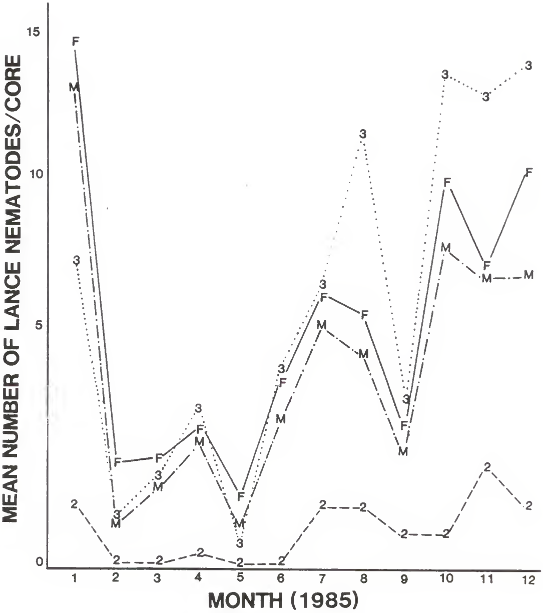

Figure 6-2. Numbers of four lance nematode life stages summed over three depths. Site two.

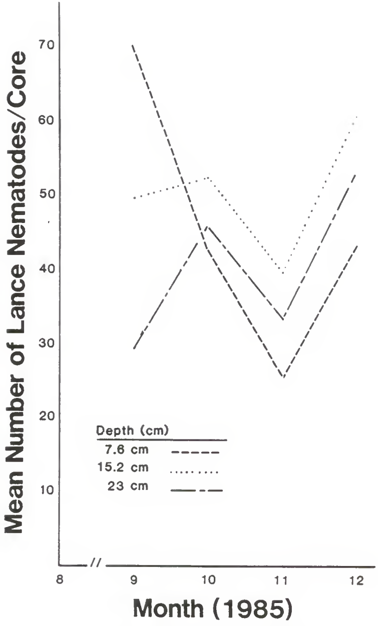

Figure 6-3. Numbers of lance nematodes per core at three depths in site one as a function of sampling date.

CHAPTER VII
TESTING THE SAMPLING PLAN

Introduction

A sampling design is only as effective as the data on which it is based. Hence, a sound sampling design should be founded on data from different sites containing a wide range of population parameters. This is especially true of the variance to mean relationship of Taylor (1961) when it is used to calculate sample size (McSorley et al., 1985).

The use of Taylor's power law to estimate population dispersion for survey or advisory purposes is helpful since it makes no assumption (requires no prior knowledge) concerning the underlying distribution of the population (Taylor, 1984). It is also simple to employ the power law to determine required sample sizes (McSorley et al., 1985; Ruesink, 1980) when given the mean population density for a given economic threshold (Ferris, 1984a, b). The slope of this relationship is also useful as a descriptor of the organism's dispersion (Taylor, 1961) and can be used to compare different organisms.

The objectives of this study were to test the sampling plan designed earlier (Chapter V) for *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935 populations in different St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] locations and times of the year, to determine if *H. galeatus* conforms to the variance-mean relationship described by Taylor, and if so, whether its life stages have similar dispersions, and to determine whether this relationship can be used to find useful sample sizes for survey and advisory purposes.

Materials and Methods

Six variously managed St. Augustinegrass sites in different Florida locations were sampled over two years (Table 7-1). Each site was divided into strata on the basis of visible surface criteria using such features as topography, weed density and type, and the density, color, vigor, and growth characteristics of the turf.

The efficiency of stratification for each of the six sites was determined using the design effect (Cochran, 1977). This is the ratio of the variance obtained from stratified random sampling to the variance which would have been obtained if the site had been randomly sampled (Cochran, 1977):

$$\text{Design effect} = \frac{1/N^2 \quad \Sigma \dfrac{N_H \quad (N_H - n)}{n_H} \quad S_H{}^2}{\dfrac{(N - n)}{nN} \quad S^2}$$

Where:

N = possible number of samples.

N_H = number of samples possible within a given stratum.

n = actual number of samples taken.

n_H = number of samples taken within the stratum.

S^2 = overall variance of the site.

S_H^2 = variance within a given stratum.

Samples were taken randomly within each stratum on the basis of its surface area. Two were taken from any area $\leq 4m^2$. Each sample consisted of a single 2.2-cm-d x 23.0 cm deep core which was bagged and processed separately (Chapter IV). The sampling intensity was targeted at the 0.25 confidence level for all soil dwelling lance nematodes (Chapter V).

The numbers of four *H. galeatus* life stages [second stage juveniles (J2), third and fourth stage juveniles (J34), males, females, and their totals] from both the soil and the roots were tallied separately. The sum of both soil and root counts is called 'all'. Some sites, especially those sampled early in the year, had few *H. galeatus* in the roots.

For each *H. galeatus* life stage (soil, root, and their sums), the number of samples which was required to estimate its density at four levels of confidence was determined. Cochran's (1977) sample size determinate for stratified random sampling ignoring stratum sampling costs was used (Chapter V); Cochran, 1977:

$$n = \frac{n_0}{1 + (n_0/N)}$$

$$n_0 = \frac{W_H \; s_H^2}{V}$$

Where:

N = number of potential samples in the site.

W_H = ratio of potential stratum samples (N_H) to potential site samples. (N_H/N).

s_H^2 = the estimation of the population standard deviation within the stratum.

V = specified level of precision based on the stratified mean ($\bar{x}_{ST}$), $((D\,\bar{x}_{ST})/t)^2$.

These sample sizes were compared to estimated sample sizes which would have been obtained if the sites had been randomly sampled.

For each site, the logs of the stratum variances were plotted against the logs of the stratum means and the parameters of Taylor's power law (Taylor, 1961) determined for each life stage and their sums. Two summary analyses were performed on the data: 1) Site differences were ignored, and the variances and means of strata were used to calculate the power law parameters; or 2) strata were ignored, and site variances and means were used.

For separate life stages, the power law parameters obtained from strata, ignoring sites, were compared to each other to determine if the hypothesis of their similarity could be supported. The power law parameters from site 6 were used to calculate random sample size, (McSorley et al., 1985; Ruesink, 1980) for the individual strata and compared to the sample size calculated using actual strata variances and optimally allocating the samples (Cochran, 1977). The formula to determine sample size using Taylor's power law is given as follows (McSorley et al., 1985; Ruesink, 1982):

$$n = \left(\frac{t}{D}\right)^{2} a\bar{x}^{(b-2)}$$

Where:

t = Approximation of the standard normal variate which at the α=0.05 confidence level will have a minimum value of 1.96 but is dependent upon the sampling intensity and is often approximated by 2.

D = ratio of the half-width of the confidence interval to the mean

a,b = parameters of Taylor's power law.

For both formulas, the confidence interval half-width to mean ratio of 25% was used to specify reliability (Karandinos, 1976; McSorley et al., 1985; Ruesink, 1982).

Results

Table 7-1 summarizes the major characteristics of the six sites. These include the sampling date, site location, maintenance level, and the range of the average H. galeatus densities and variations encountered in the strata for that site.

Stratification on the basis of surface criteria proved effective (Table 7-2) for all soil dwelling H. galeatus life stages, their total, and the total of soil and root dwelling lance nematodes for five of the six sites studied. The exception was one site (site 5) which exhibited a markedly uniform density throughout the small lawn (Table 7-1). Stratification was less effective for root dwelling lance nematodes.

Sampling intensities for three levels of precision and the various life stages of H. galeatus, calculated as shown on page 106, are presented in Table 7-3. The sampling intensity calculated earlier (Chapter V) for the 0.25 confidence level of total soil dwelling lance nematodes (Table 5-8, n=44) overestimated the sampling intensity for five of six sites by at least 13 samples. This same number of cores (n = 44) was sufficient to meet the demands of the 0.20 confidence level for all but two sites.

Table 7-4 compares the sample sizes for stratified random sampling and random sampling at two levels of precision for the six sites. Fewer samples were required when the site was stratified using visible surface criteria.

Parameters of Taylor's power law for each soil dwelling life stage, the sum of all stages from soil, and the sum of both soil and root dwelling life stages are presented in Table 7-5. Except for site four, a fair relationship between the variance and the mean was observed.

Combined site analyses are presented in Table 7-6. The variance to mean relationship continued to hold for individual strata, ignoring sites. A comparison, by creation of a dummy variable (SAS Institute Inc., 1982c), of Taylor's power law dispersion indices for the various soil dwelling life stages supported the hypothesis of their similarity was. The summary analysis exploring sites, ignoring strata, explained little variation, but was better for the soil dwelling juveniles or root dwelling life stages than for others (Table 7-6).

The low coefficients of determination for Taylor's power law parameters obtained from combined data, ignoring strata (Table 7-6), did not allow a comparison of estimated sample sizes between stratified random sampling, random sampling and random sampling as determined with Taylor's power law. Table 7-7 presents a similar comparison for strata in site six, using power law parameters calculated from the site (Table 7-5). The sum of these estimated random sample sizes as calculated by the power law (n = 193, Table 7-7) and the sample size as determined from the variance which would have been obtained if the site had been randomly sampled (n = 190, Table 7-4) agree fairly closely. Although fewer samples were required when the site was stratified, optimal allocation called for less than one sample in a number of strata (Table 7-7, Stratified allocation - real), a physical impossibility. When sample numbers from each stratum were rounded up to the nearest whole number, the actual number of cores must increase by six, from 40 to 46.

Discussion

Stratification on the basis of visible surface features seems to be an effective strategy for sampling soil dwelling *H. galeatus* associated with St. Augustinegrass. Stratification seemed to be most worthwhile in the two high population, high maintenance sites (Table 7-1, 7-2). Although stratification reduced by ca. 20-50% the expected variation from randomly sampling root dwelling lance nematodes (Table 7-2), it did not reduce it at all in site five, or for all root dwelling life stages. Stratification was most effective for the root dwelling J34 life stage, which was the predominant life stage recovered from the roots (see also Chapter VI). The relatively high density of this root dwelling group compared to the others strongly contributed to the overall efficiency of stratification for all root dwelling life stages.

Both high maintenance sites were difficult to stratify because they offered few visual cues. Irregularities in turf growth, appearing similar to "frost boils", imperfectly distinguished only the highest nematode densities. These difficulties in stratification may occur because plants adequately supplied with nutrients and water seem to tolerate rather high populations of H. galeatus (Krusberg and Sasser, 1956). High maintenance levels may mask the differential effects of factors that might have permitted more effective stratification, leading to an increase in sample size.

The sampling of six sites with the plan designed in Chapters IV and V indicates that the required sample size for estimating total soil lance nematode populations at a 25% level of precision (n = 44) was an overestimate, by at least 13 samples in five of six sites, and was sufficient to estimate the populations with 20% confidence in four of the six sites.

The four observed life stages of H. galeatus and their totals showed fair agreement with the log variance-log mean relationship described by Taylor (1961) for five of the six sites studied. The similarities in the β values of the different life stages confirm an earlier observation (Chapter V) for a single site.

The lack of fit of site variances and means to Taylor's power law parameters is probably due both to the small sample size (n=6) and the variability of these areas when strata are eliminated. Increasing the number of sites sampled would probably provide a better fit to the variance-mean relationship and better estimation of sample size.

The site data presented here and in Table 7-7 offer some general guidelines for the construction and sampling of strata. Stratification is most efficient when the population densities within a site vary greatly and these differences can be related to some indication as to their distribution. More samples need to be taken from strata which have the greatest internal variation, and from larger strata, especially those in moderately-high to high maintenance sites where internal variation in the strata is likely to be greatest. When resampling an area using optimal allocation, strata with low variability and with small sample size requirements might be merged to minimize actual sample numbers.

The current threshold level recognized by the University of Florida Nematode Assay Lab for _H_. _galeatus_ associated with St. Augustinegrass is 40/100 cm^3 of soil (Dunn, 1984).

Given a confidence of 25%, about 30 cores should be drawn to estimate an area of low to moderate maintenance thought to be infested with H. galeatus. Forty or more cores should be drawn from lawns receiving moderately-high or high maintenance.

It would help if these cores were taken in accordance with stratification procedures, especially since these sample size estimates are based on data which have incorporated stratification data in them. For a person accustomed to working with lawns or the homeowner who watches it daily, stratification should be fairly easy and could enhance the reliability of the estimate. It is unlikely, however, that many homeowners or pest control operators will take as many samples as necessary for any but low levels of confidence.

Table 7-1. Description of the six St. Augustinegrass sites sampled for this study.

				Range in stratum	
Site	Sampled	Location	Level	means ($\bar{x}$)	variances (s^2)
1	Sept., 1986	central Florida	low	24.2- 89.7	244.1- 4496.6
2	May, 1985	north-central Florida	low	6.5- 19.0	37.2- 330.0
3	April, 1985	north-central Florida	moderate	4.8-149.7	329.4- 1286.3
4	Oct., 1986	central Florida	moderately-high	37.5-213.3	1105.3-23857.3
5	April, 1986	southeast Florida	moderate	16.2- 16.5	78.6- 215.0
6	Oct., 1986	central Florida	high	4.7-209.7	57.7- 7570.3

Table 7-2. Gain due to stratification in six study sites as calculated by the design effect for Hoplolaimus galeatus life stages in the soil and in the roots.

	Gain due to stratificatioln by site[Y]					
Life stages	1	2	3	4	5	6
Soil						
J2	.73	.72	.62	.65	.75	.21
J34	.65	.57	.55	1.0	1.0	.41
Male	.59	.61	.56	.47	1.0	.24
Female	.62	.58	.52	.48	1.1	.29
Total	.62	.59	.52	.61	1.1	.28
Root						
J2	1.0	--	--	--	--	--
J34	.11	.79	.77	1.8	--	.50
Male	1.0	.1	.79	.55	--	1.4
Female	1.1	.88	.78	4.0	--	1.2
Total	1.1	.79	.71	.82	--	.81
All[Z]	.68	.29	.53	.73	--	.21

[Y] Design Effect = $\frac{\text{Variance from stratified random sampling}}{\text{Variance from random sampling}}$;

[Z] All = both soil and root dwelling forms, counted for all life stages.

Table 7-3. Sample size determination for three confidence levels in six study sites. *Hoplolaimus galeatus* life stages in the soil and soil plus roots (all).

H. galeatus life stage	Confidence[Y] level	Site[z] 1	2	3	4	5	6
J2	.1	2	3	3	9	4	1
	.2	1	2	2	2	2	1
	.25	1	1	1	2	2	1
J34	.1	38	35	101	22	34	32
	.2	10	9	25	6	9	8
	.25	6	6	7	4	6	5
Male	.1	9	20	136	7	9	28
	.2	2	5	34	2	3	7
	.25	2	4	10	2	2	5
Female	.1	21	89	201	18	13	28
	.2	6	22	51	5	4	7
	.25	4	15	14	3	2	5
Total	.1	150	167	1138	152	82	226
	.2	38	41	285	38	22	57
	.25	24	27	78	25	14	31
All	.1	160	167	1329	153	82	246
	.2	40	41	333	38	22	62
	.25	26	27	91	25	14	40

[Y] Half-width of the confidence interval to mean.

[z] The number of samples is rounded up to the nearest whole number.

Table 7-4. Comparison of random and stratified random sample sizes for summed soil and root populations in six sites.

Site	Sample size by type and confidence level (D)[z]			
	Stratified sampling		Random sampling	
	D		D	
	.1	.25	.1	.25
1	160	26	236	39
2	167	27	576	94
3	1329	91	2508	314
4	153	25	210	35
5	82	14	78	13
6	246	40	1172	190

[z] D = ratio of half-width of the confidence interval to the mean.

Table 7-5. Parameters of Taylor's power law (α, β) relating the variance to the mean and the coefficient of determination of the model (R^2) for four soil dwelling life stages of Hoplolaimus galeatus, total of all soil life stages, and for both soil and root dwelling sums (all).

Site	J2	J34	Male	Female	Total	All
1						
α	0.80	-0.77	-0.23	-0.62	.71	-1.89
β	1.428	2.082	1.834	1.502	1.925	2.213
$R^{2,z}$	.94***	.98****	.95***	.73*	.96***	.70*
2						
α	0.12	0.75	0.18	0.40	0.64	0.74
β	1.078	0.51	1.280	1.376	1.262	1.170
R^2	.96***	.23	.94***	.92***	.76*	.78*
3						
α	1.0	2.79	2.80	2.88	3.66	3.72
β	1.04	1.117	1.179	1.159	1.158	1.154
R^2	.88*	.96**	.94*	.93*	.92*	.92*
4						
α	1.09	1.46	1.52	1.42	2.57	2.68
β	1.182	1.330	1.088	1.241	1.191	1.164
R^2	.03	.63**	.24	.23	.39*	.13
5						
α	--	0.81	0.51	0.93	0.34	0.55
β	--	1.46	0.4	1.52	1.40	1.120
R^2	--	.98***	.14	.92***	.93***	.70*
6						
α	-.12	0.99	1.10	1.40	1.65	0.92
β	0.980	1.505	1.520	1.292	1.422	1.522
R^2	.97****	.85****	.97****	.71***	.80****	.84****

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[z] Coefficients of determination (R^2) followed by *, **, ***, or **** are significant at the 0.05, 0.01, 0.001, or 0.0001 levels, respectively.

Table 7-6. Parameters of Taylor's power law (α, β) and coefficients of determination (R^2) for soil and root dwelling life stages and their totals, for summary data retaining strata within sites or ignoring strata and using sites alone.

	J2	J34	Male	Female	Total	All
Retaining strata, soil dwelling						
α	0.82	1.4	1.31	1.79	2.19	1.32
β	1.108	1.364	1.302	1.162	1.285	1.465
R^2,[z]	.95****	.85****	.79****	.66****	.77****	.76****
Sites alone, ignoring strata, soil dwelling						
α	1.3	3.82	3.89	4.2	5.2	5.4
β	1.516	0.822	0.73	0.691	0.780	0.716
R^2	.97**	.92*	-.10	.13	.35	.12
Retaining strata, root dwelling						
α	--	.62	0.05	.29	.63	
β	--	1.075	1.005	1.03	1.072	
R^2	--	.98****	.97****	.99****	.98****	
Sites alone, ignoring strata, root dwelling						
α	--	1.24	0.29	0.27	1.13	
β	--	1.363	1.061	0.90	1.372	
R^2	--	.80*	.89*	.41	.64	

---

[z] Coefficients of determination (R^2) followed by *, **, or **** are significant at the 0.05, 0.01, or 0.0001 levels, respectively.

Table 7-7. Comparison of site six stratum sample sizes as determined through allocation for stratified random sampling with optimal allocation and for random sampling using the parameters of Taylor's power law obtained from the site.

		Sample size		
		Stratified allocation[Y]		
Stratum	Stratum means	Real	Actual	Random, by Taylor's power law[Z]
1	19.5	.91	1	15
2	29.5	2.2	3	12
3	6.7	.29	1	24
4	8.5	.40	1	22
5	13.7	1.15	2	17
6	4.7	.03	1	28
7	9.0	.74	1	21
8	33.8	.56	1	11
9	73.8	12.00	12	8
10	23.3	1.54	2	13
11	99.7	4.50	5	7
12	48.0	7.00	7	10
13	209.7	9.00	9	5
		39.9	46	193

[Y] Stratum sample size (n_H) = $((W_H s_H^2)/(W_H s_H^2))n$
where: n = number of samples drawn from the site.
s_H^2 = stratum variance.
W^H = N_H/N, the number of potential samples in the stratum and the site, respectively.

[Z] $(2/.25)^2 \ .92(\overline{x}_{st})^{(2 - 1.522)}$
where: $\overline{x}_{st}$ = stratum mean.

CHAPTER VIII
SUMMARY AND CONCLUSIONS

Hoplolaimus galeatus reproduced similarly on seven commonly-available varieties of St. Augustinegrass in two greenhouse and one microplot trial, indicating similar host suitabilities. Significant but low correlations were seen between the numbers of H. galeatus in the soil and in the roots. There was a trend toward reduced clipping weights of inoculated plants with increased time after infestation. At the final cutting time, the Roselawn-Floratine group produced more than did the Bitterblue group. No cutting weight differences were observed between diploid and triploid varieties. Reproduction of Paratrichodorus spp., present as a minor contaminant in the outdoor microplot trial, indicates that St. Augustinegrass varieties may react differently to this nematode.

Extraction losses and core size recovery of two differently sized sampling units were examined. Nematodes from the larger sampling unit, 10.2-cm-d, were more difficult to extract efficiently than nematodes in a small sampling unit, 2.2-cm-d. When processing the larger sample unit by a modified sieving centrifugation extraction procedure, greatest losses of nematodes occurred in the soil pellet remaining in the tube after centrifugation; next greatest losses were in the soil fraction discarded after the initial suspension and sieving. The greatest loss of nematodes when processing the 2.2-cm-d sampling unit occurred when the water supernatant was discarded from the centrifuge tube.

Despite large losses associated with processing the 10.2-cm-d sampling unit of field samples, a greater percentage of *H. galeatus* was recovered by the large sampling unit than by the small when processing artificially infested soil. This result may reflect more on the mixing process by which the nematodes were incorporated into the soil than on recovery. Incomplete mixing of the nematode solution into the soil might result in their being deposited in clumps. In this instance, a small sample unit would encounter few clumps and recover more zero observations than expected. A large sample unit would be expected to encounter many more clumps.

A study was conducted to determine an optimal sampling strategy, using a stratified random design with proportional allocation on the basis of surface area. Two sampling units were used to sample three depths, 0-7.6, 7.7-15.2, and 15.3-23.0 cm. Stratifying a St. Augustinegrass site on the basis of surface characteristics reduced the variation which would have been expected from simple random sampling anywhere from 0-50%.

Differences in the vertical distribution of *H. galeatus* were also seen. The 10.2-cm-d sampling unit differentiated between the densities at the two deeper depths whereas the small sample unit usually did not. Fewest _H_. _galeatus_ were found in the 0-7.6-cm depth while the most were usually recovered from the deepest depth sampled.

Chi-square analysis showed the large sampling unit captured proportionally more _H. galeatus_ than did the small sampling unit. Fewer zeros and more high density observations resulted with this sampling unit. Consequently, fewer large samples than small samples were required for the same degree of precision. However, the large sampling unit was discarded from further consideration because of its large extraction losses and the amount of time required for processing.

Two St. Augustinegrass sites, one with a high and the other with a low population density, were sampled monthly throughout the year at three depths. Highest population densities occurred in July and September-October, periods which seem to correspond to the most active growth of St. Augustinegrass. At these same periods, fewer samples were required to estimate the population than at other times. Maximal specific growth rate of _H_. _galeatus_ was about 4.3 and occurred in May-June. Investigation of age structure showed that adult _H_. _galeatus_ are relatively long-lived, and late spring or early summer increases depend on an increase in juveniles in January through April. The best time to apply a nematicide for _H_. _galeatus_ therefore appears to be January through April.

More nematodes occurred in the upper soil layers during the spring and summer months than at the 15.2-23.0-cm depths. Around December, however, a density shift occurred and fewest were found in the 0-7.6-cm layer. These shifts may correspond to colder temperatures.

Both *H. galeatus* populations seemed to be reduced by a series of heavy, flooding rains in August. Other factors which seemed to influence their populations as judged by multiple linear regression were days of rain and photosynthetically active radiation.

Stratified random sampling of six sites throughout the state at different times over two years was used to test the sampling design. Stratification on the basis of surface criteria improved reliability, especially in high maintenance sites. Sample size for a 25% confidence level was overestimated in the design study by at least 13 samples. All life stages of *H. galeatus* examined had an identical dispersion parameter as determined by Taylor's power law.

LITERATURE CITED

Ahmad, M., and T. A. Chen. 1980. Effect of certain environmental factors and host plants on reproduction of Hoplolaimus galeatus. Plant Disease 64:479-480.

Alby, T., J. M. Ferris, and V. R. Ferris. 1983. Dispersion and distribution of Pratylenchus scribneri and Hoplolaimus galeatus in soybean fields. Journal of Nematology 15:418-426.

Andrassy, I. 1958. Hoplolaimus tylenchiformis Daday, 1905 (syn. H. coronatus Cobb, 1923) und die Gattungen der Unterfamilie Hoplolaiminae Filipjev, 1936. Nematologica 3:44-46.

Andrassy, I. 1962. The problem of number and size of sampling unit in quantitative studies of soil nematoda. Pp. 65-67 in P.W. Murphy, ed. Progress in soil zoology. London: Butterworth's.

Anscombe, F. J. 1950. Soil sampling for potato root eelworm cysts. Annals of Applied Biology 37:286-295.

Antonovics, J. 1984. Genetic variation within populations. Pp. 229-241 in R. Dirzo and J. Sarukhan, eds. Perspectives on plant population biology. Sunderland, MA: Sinauer Associated Inc.

Ayala, A., J. Roman, and A. C. Tarjan. 1963. Comparison of four methods for isolating nematodes from soil samples. Journal of Agriculture of the University of Puerto Rico 47:219-225.

Ayoub, S. K. 1980. Plant nematology: an agricultural training aid. Sacramento: NemaAid Publications.

Barker, K. R. and C. J. Nusbaum. 1971. Diagnostic and advisory programs. Pp. 281-301 in B. M. Zuckerman, W. F. Mai and R. A. Rhode, eds. Plant Parasitic Nematodes, Vol. I. New York: Academic Press.

Barker, K. R., C. J. Nusbaum, and L. A. Nelson. 1969a. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. Journal of Nematology 1:232-239.

Barker, K. R., C. J. Nusbaum, and L. A. Nelson. 1969b. Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. Journal of Nematology 1:240-247.

Beard, J. 1982. A new perspective on root growth. Proceedings of the Florida Turf-Grass Conference 30:24-32.

Boag, B., and P. B. Topham. 1984. Aggregation of plant parasitic nematodes and Taylor's power law. Nematologica 30:348-357.

Burns, N. C. 1971. Soil pH effects on nematode population associated with soybeans. Journal of Nematology 3:238-245.

Busey, P. 1980. Gamma ray dosage and mutation breeding in St. Augustinegrass. Crop Science 20:181-184.

Busey, P., and B. J. Augustin. 1980. Appraisal of St. Augustinegrasses for south Florida. Proceedings of the Florida State Horticultural Society 93:99-102.

Busey, P., T. K. Broschat, and B. J. Center. 1982. Classification of St. Augustinegrass. Crop Science 22:469-473.

Busey, P., and E. O. Burt. 1979. Turf, energy, and the environment. Proceedings of the Florida State Horticulture Society 92:224-227.

Busey, P., and B. J. Myers. 1979. Growth rates of turf grasses propagated vegetatively. Agronomy Journal 71:817-821.

Chapman, R. A. 1976. Population dynamics of Hoplolaimus galeatus in sod. Journal of Nematology 8:282 (Abstr.).

Christie, J. R., J. M. Good, Jr., and G. C. Nutter. 1954. Nematodes associated with injury to turf. Proceedings of the Soil Science Society of Florida 14:167-169.

Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Proceedings of the Helminthological Society of Washington 18:106-108.

Church, B. M., H. C. Gough, and J. F. Southey. 1959. Soil sampling procedures for potato root eelworm cysts. Plant Pathology 8:146-151.

Cobb, N. A. 1918. Estimating the nema population of soil. Washington: Agricultural Technology Circular 1, USDA, Bureau of Plant Industry, Office of Technology.

Cobb, N. A. 1923. An Amendation of Hoplolaimus Daday, 1905, nec auctores. Journal of the Washington Academy of Science 13:211-214.

Cochran, W. G. 1977. Sampling Techniques. New York: John Wiley and Sons.

Coomans, A. 1962. The spicular muscles in males of the subfamily Hoplolaiminae (Tylenchida, Nematoda). Biologisch Jaarboek 12:313-315.

Dasgupta, D. R., S. Nand, and A. R. Seshadri. 1970. Culturing, embryology and life history studies on the lance nematode, Hoplolaimus indicus. Nematologica 16:235-248.

Dunn, R. A. 1984. Turf nematodes: Diagnosis and control for turf maintenance professionals. Nematology Plant Protection Pointer 18. Gainesville, FL: University of Florida.

Endo, B. Y. 1959. Responses of root-lesion nematodes, Pratylenchus brachyurus and P. zeae, to various plants and soil types. Phytopathology 49:417-421.

Falk, J. H. 1976. Energetics of a suburban lawn ecosystem. Ecology 57:141-150.

Fassuliotis, G. 1975. Feeding, egg-laying, and embryology of the Columbia lance nematode, Hoplolaimus columbus. Journal of Nematology 7:152-158.

Ferris, H. 1984a. Nematode damage functions: the problems of experimental and sampling error. Journal of Nematology 16:1-9.

Ferris, H. 1984b. Probability range in damage predictions as related to sampling decisions. Journal of Nematology 16:246-251.

Fidler, J. H., B. M. Church, and J. F. Southey. 1959. Field sampling and laboratory examination of cereal root eelworm cysts. Plant Pathology 8:27-34.

Finch, S., G. Skinner, and G. H. Freeman. 1975. The distribution and analysis of cabbage root fly egg populations. Annals of Applied Biology 79:1-18.

Francl, L. J. 1986. Improving the accuracy of sampling field plots for plant-parasitic nematodes. Journal of Nematology 18:190-195.

Good, J. M., J. R. Christie, and J. C. Nutter. 1956. Identification and distribution of plant parasitic nematodes in Florida and Georgia. Phytopathology 46:13 (Abstr.).

Goodell, P. B. 1982. Soil sampling and processing for detection and quantification of nematode populations for ecological studies. Pp. 178-198 in D. W. Freckman, ed. Nematodes in soil ecosystems. Austin: University of Texas Press.

Goodell, P., and H. Ferris. 1980. Plant-parasitic nematode distributions in an alfalfa field. Journal of Nematology 12:136-141.

Goodell, P. B., and H. Ferris. 1981. Sample optimization for five plant-parasitic nematodes in an alfalfa field. Journal of Nematology 13:304-313.

Green, C. D., and D. M. Parrott. 1966. A re-appraisal of the extraction of Heterodera cysts from large volumes of soil. Nematologica 12:601-609.

Gupta, J. C., and A. S. Atwal. 1971. Biology and ecology of Hoplolaimus indicus (Hoplolaiminae: Nematoda). I. The life stages and the feeding behaviour. Nematologica 17:69-74.

Harrison, J. M., and C. D. Green. 1976. Comparison of centrifugal and other methods for standardization of extraction of nematodes from soil. Annals of Applied Biology 82:299-308.

Hirschmann, H. 1959. Histological studies on the anterior region of Heterodera glycines and Hoplolaimus tylenchiformis (Nematoda, Tylenchida). Proceedings of the Helminthological Society of Washington 26:73-90.

Hogger, C., and G. W. Bird. 1974. Secondary male sex characteristics of Hoplolaimus galeatus. Journal of Nematology 6:12-16.

Hutchinson, M. T., J. P. Reed, and D. Pramer. 1960. Observations on the effects of decaying vegetable matter on nematode populations. Plant Disease Reporter 44:400-401.

Hutton, D. G. 1978. Influence of rainfall on some plantain nematodes in Jamaica. Nematropica 8:34-39.

Jackson, R. C. 1976. Evolution and systematic significance of polyploidy. Annual Review of Ecology and Systematics 7:209-234.

Jefferies, R. L. 1984. The phenotype: its development, physiological constraints, and environmental signals. Pp. 347-358 in R. Dirzo and J. Sarukhan, eds. Perspectives on plant population biology. Sunderland, MA: Sinauer Associated Inc.

Jones, F. G. W. 1975. Accumulated temperature and rainfall as measures of nematode development and activity. Nematologica 21:62-70.

Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bulletin of the Entomological Society of America 22:417-421.

Kelsheimer, E. G., and A. J. Overman. 1953. Notes on some ectoparasitic nematodes found attacking lawns in the Tampa Bay area. Proceedings of the Florida State Horticultural Society 66:301-303.

Krusberg, L. R., and J. N. Sasser. 1956. Host-parasite relationships of the lance nematode in cotton roots. Phytopathology 46:505-510.

Little, T. M. 1985. Analysis of percentage and rating scale data. Hort Science 20:642-644.

Lucas, L. T., K. R. Barker, and C. T. Blake. 1978. Seasonal changes in nematode densities on bentgrass golf greens in North Carolina. Plant Disease Reporter 62:373-376.

McSorley, R., W. H. Dankers, J. L. Parrado, and J. S. Reynolds. 1985. Spatial distribution of the nematode community on perrine marl soils. Nematropica 15:77-92.

McSorley, R., and J. L. Parrado. 1981. Effect of sieve size on nematode extraction efficiency. Nematropica 11:165-174.

McSorley, R., and J. L. Parrado. 1982a. Estimating relative error in nematode numbers from single soil samples composed of multiple cores. Journal of Nematology 14:522-529.

McSorley, R., and J. L. Parrado. 1982b. Plans for the collection of nematode soil samples from fruit groves. Nematropica 12:257-267.

Metcalf, R. L., and W. H. Luckmann, eds. 1975. Introduction to insect pest management. New York: John Wiley & Sons.

Minton, N. A., E. J. Cairns, and A. L. Smith. 1960. Effect on root-knot nematode populations of resistant and susceptible cotton. Phytopathology 50:784-787.

Ng, O. -C., and T. A. Chen. 1985. The histopathology of alfalfa roots infected by Hoplolaimus galeatus. Phyto pathology 75:297-304.

Norton, D. C. 1979. Relationship of physical and chemical factors to populations of plant-parasitic nematodes. Annual Review Phytopathology 17:279-299.

Nutter, G. C. 1958. Progress in nematode research. Proceedings University of Florida Turf Management Conference 6:101-108.

Parris, G. K. 1957. Screening Mississippi soils for plant parasitic nematodes. Plant Disease Reporter 41:705-706.

Peacock, C. H. 1981. Effects of water stress on photosynthesis and evapotranspiration in St. Augustinegrass, Stenotaphrum secundatum (Walt.) Kuntze. Ph.D. dissertation, University of Florida, Gainesville.

Perry, V. G. 1953. Return of nematodes following fumigation of Florida soils. Proceedings of the Florida State Horticultural Society 66:112-114.

Perry, V. G., and J. M. Good. 1968. The nematode problems of turfgrasses. Proceedings of the Florida Turf-Grass Management Conference 16:22-25.

Perry, V. G., G. C. Smart, Jr., and G. C. Horn. 1970. Nematode problems of turfgrasses in Florida and their control. Proceedings of the Florida State Horticultural Society 83:489-492.

Perry, V. G., and J. A. Winchester. 1969. Developments in nematode control. Proceedings of the Florida Turf-Grass Management Conference 17:88-90.

Rajaram, B. 1983. Biology of the lance nematode Hoplolaimus seinhorsti Luc, 1958. European Society of Nematologists 16 International Symposium: 54 (Abstr.).

Reinert, J. A., and A. E. Dudeck. 1974. Southern chinch bug resistance in St. Augustinegrass. Journal of Economic Entomology 67:275-277.

Rivera-Camarena, J. E. 1963. Pathogenic and biological aspects of sting and lance nematodes. Ph.D. dissertation, University of Florida, Gainesville.

Rodriguez-Kabana, R., and P. S. King. 1975. Efficiency of extraction of nematodes by flotation-sieving using molasses and sugar and by elutriation. Journal of Nematology 7:54-59.

Ruesink, W. G. 1980. Introduction to sampling theory. Pp. 61-78 in M. Kogan and D.C. Herzog, eds. Sampling methods in soybean entomology. New York: Springer-Verlag.

Ruesink, W. G., and M. Kogan. 1975. Quantitative basis of pest management: sampling and measuring. In: Metcalf, R. L. and W. H. Luckmann (eds.) Introduction to pest manage ment. New York: John Wiley & Sons.

SAS Institute Inc. 1982a. SAS user's guide: basics. Cary, NC: SAS Institute Inc.

SAS Institute Inc. 1982b. SAS user's guide: statistics. Cary, NC: SAS Institute Inc.

SAS Institute Inc. 1985. SAS for linear models: a guide to the ANOVA and GLM procedures. Cary, NC: SAS Institute Inc.

Sauer, J. D. 1972. Revision of Stenotaphrum (Gramineae: Paniceae) with attention to its historical geography. Brittonia 24:202-222.

Schiel, J. B., Jr. 1971. The ecology and distribution of plant-parasitic nematodes on three farms in southern Illinois. Ph.D. dissertation, University of Illinois, Urbana-Champaign.

Sher, S. A. 1961. Revision of the Hoplolaiminae (Nematoda) I. Classification of nominal genera and nominal species. Nematologica 6:155-169.

Sher, S. A. 1963. Revision of the Hoplolaiminae (Nematoda) II. Hoplolaimus Daday, 1905 and Aorolaimus N. Gen. Nematologica 9:267-295.

Snaydon, R. W. 1984. Plant demography in an agricultural context. Pp. 389-407 in R. Dirzo and J. Sarukhan, eds. Perspectives on plant population biology. Sunderland, MA: Sinauer Associated Inc.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Ames: Iowa State University Press.

Southwood, T. R. E. 1978. Ecological methods with particular reference to the study of insect populations. New York: Halsted Press.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189:732-735.

Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. Annual Review of Entomology 29:321-357.

Taylor, L. R., R. A. J. Taylor, I. P. Woiwod, and J. N. Perry. 1983. Behavioural dynamics. Nature 303:801-804.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. Journal of Ecology 63:995-1001.

Thistlethwayte, B., and R. M. Riedel. 1969. Expressing sucrose concentrations in solutions used for extracting nematodes. Journal of Nematology 1:387-388.

Viglierchio, D. R., and R. V. Schmitt. 1983a. On the methodology of nematode extraction from field samples: Baermann funnel modification. Journal of Nematology 15:438-444.

Viglierchio, D. R., and R. V. Schmitt. 1983b. On the methodology of nematode extraction from field samples: comparison of methods for soil extraction. Journal of Nematology 15:450-454.

Viglierchio, D. R., and T. Yamashita. 1983. On the methodology of nematode extraction from field samples: Density flotation techniques. Journal of Nematology 15:444-449.

Wallace, H. R. 1978. Nematode ecology and plant disease. New York: Edward Arnold.

Whitton, G. M., Jr. 1956. Aspects of the nematode problem. Proceedings University of Florida Turf Management Conference 4:99-102.

Widdowson, E. 1962. The estimation of soil populations of _Heterodera_ _rostochiensis_ Woll. Pp. 59-64 _in_ P. W. Murphy, ed. Progress in soil zoology. London: Butterworth's.

Willut, J. M., and R. B. Malek. 1981. Population response to temperature by _Hoplolaimus_ _galeatus_, _Helicotylenchus_ _pseudorobustus_, and _H_. _dihystera_. Journal of Nematology 13:463 (Abstr.).

Yates, F., and D. J. Finney. 1942. Statistical problems in field sampling for wireworms. Annals of Applied Biology 29:156-167.

Yeates, G. W. 1981, Nematode populations in relation to soil environmental factors: a review. Pedobiologia 22:312-338.

BIOGRAPHICAL SKETCH

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He married in 1986.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Robert A. Dunn,
Chairman
Professor of Entomology
and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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